1994

Muscle development, energy source utilization and metabolism hormone activity in Colorado potato beetle, Leptinotarsa decemlineata (Say) flight.

Bin Yang
University of Massachusetts Amherst

MUSCLE DEVELOPMENT, ENERGY SOURCE UTILIZATION AND METABOLISM HORMONE ACTIVITY IN COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY) FLIGHT

A Thesis Presented

by

BIN YANG

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements of the degree of

MASTER OF SCIENCE

September, 1994

Entomology

MUSCLE DEVELOPMENT, ENERGY SOURCE UTILIZATION AND METABOLISM HORMONE ACTIVITY IN COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY) FLIGHT

A Thesis Presented

by

BIN YANG

Approved as to style and content by:

David N. Ferro, Chair

Chih-Ming Yin, Member

John H. Nordin, Member

Ring T. Cardé, Department Head
Entomology

ACKNOWLEDGMENTS

I would like to acknowledge Dr. David Ferro for giving me the opportunity to work on this project. I would also like to express my appreciation to Dr. Chih-Ming Yin. Most of this project was done in Dr. Yin's lab, without his guidance and encouragement, this thesis would have been impossible to finish. Also my committee member Dr. John Nordin provided a lot help, his door always kept open for me.

I also thank people in Ferro's lab (Don Weber, Rolando Lopez, Andy Slocombe, Chris Mercier, and Baode Wang) and people in Dr. Yin's lab (Baixiang Zou, Meifang Li, and Wenhong Qin), thanks for their generous help and friendship.

My wife Tong has always been encouraging and helpful. Her love and support made this thesis to be possible.

ABSTRACT

MUSCLE DEVELOPMENT, ENERGY SOURCE UTILIZATION AND METABOLISM HORMONE ACTIVITY IN COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY) FLIGHT

SEPTEMBER 1994

BIN YANG, B.A., BEIJING AGRICULTURAL UNIVERSITY

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor David N. Ferro

Energy source utilization during the flight and metabolism hormone activity were studied in fed and unfed post-diapause and summer generation Colorado potato beetles.

For post-diapause fed beetles, proline concentration in the hemolymph did not significantly change during the 60 minute flight, while alanine concentration increased 466.3% over the flight; and the concentrations of total carbohydrates and total lipids also decreased during the flight of 60 minutes. For post-diapause unfed beetles, proline concentration in the hemolymph significantly decreased during the flight, and alanine concentration increased 2441.9% at the same time, however, total

carbohydrates and total lipids were no different when beetles flew.

Metabolism hormones are produced in insect corpora cardiaca (CC). The CC extracts from post-diapause fed beetles and summer generation beetles mobilized alanine and total carbohydrate levels in CC injected recipient beetles. However, the CC extracts from post-diapause unfed beetles only mobilize the alanine concentration in the recipient beetles.

The flight muscle development and regeneration also were studied in fed and unfed post-diapause and summer generation Colorado potato beetles. The post-diapause beetles needed about 150-200 degree-day for completing the flight muscle regeneration, and the regeneration was only relative to accumulated heat, regardless whether the beetles remained in the soil or emerged from the soil. The flight muscles in unfed post-diapause beetles began to degenerate after 20 days emerged, while fed post-diapause beetles always maintained fully regenerated flight muscles, the flight muscles in unfed post-diapause beetles were only about 1/7 of flight muscles in fed post-diapause beetles. The flight muscles in mated and productive female beetles had no different from unmated female beetles.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW: ECOLOGY AND PHYSIOLOGY OF COLORADO POTATO BEETLE DISPERSAL

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the major insect pest of potatoes in North America and Europe. In addition to its diverse life history and insecticide resistance, its dispersal of poses a challenge to Colorado potato beetle management. The understanding of the ecology and physiology of Colorado potato beetle dispersal is very important for management programs.

General Life History

Colorado potato beetle has a very diverse and flexible life history among different geographic populations, and also within local populations. Colorado potato beetle can have 1-4 generations in one year depending on the geographic distribution (Hare, 1990). Adult Colorado potato beetle diapause in the soil, usually at a depth of between 7.6 cm and 12.7 cm

(Lashomb, 1984). In the spring when the temperature reaches 8-12 ^{o}C, the beetles become active and emerge from the soil (these beetles are named overwintered or post-diapause beetles) (Ferro *et al.*, 1985). Lashomb (1984) developed a logistic curve to predict emergence relative to accumulated degree-days. After emerging, the adults disperse by walking or flying (Voss and Ferro, 1990b). When the beetles find a suitable host, they begin feeding immediately. Five to eight days later, female beetles start to deposit a total of 500 - 1,000 eggs in masses of 20-60 eggs (Jacques, 1988; Mateeva-Radeva, 1985; Peferoen *et al.*, 1981). Usually, the egg masses are deposited on the underside of foliage. Adult beetles can mate before or after diapause and mating is multiple and polygamous (Tauber *et al.*, 1988). Under optimal conditions (25-30 ^{o}C, long-day photoperiod, and high quality food), the generation time, from egg to adult, can be as short as 21 days (Ferro *et al.*, 1985). Offspring of post-diapause beetles are named first summer generation beetles, and offspring of first summer generation beetles are called the second summer generation beetles. In western Massachusetts, the first generation beetles are the major population in the summer stage, so the first generation beetles also are called summer generation beetles.

Ecology of CPB Dispersal

Dispersal plays a major role in the life history of the Colorado potato beetle. Knowledge of Colorado potato beetle dispersal can contribute to our understanding of the basic population dynamics and to development of potential management schemes (Price *et al.*, 1975).

There are many papers in the literature on the dispersal of the Colorado potato beetle. Tower (1906) first reported on the spread of this beetle from the mid-west to the east coast of North America. Wiktelius (1981) demonstrated that Colorado potato beetle could cross the North Sea to Scandinavia. Charles (1988) studied the Colorado potato beetles movement within field and the dispersion on the host of eggplant.

Colorado potato beetle dispersal behavior varies over its geographic range. In the Netherlands, beetles were rarely observed in flight within fields, however, they were frequently seen in the English channel of the French coast (De Wilde and Hsiao, 1981). In France, most flights occurred at the end of July to mid-August, but after destruction of crops or when a harvest interrupted adults feeding, frantic flight was observed, sometimes resulting in mass aggregation of beetles against walls of buildings (De Wilde and Hsiao, 1981).

In the laboratory, Mordue and De Kort (1978) used the roundabout (a kind of flight mill) to show that beetles reared under short-day conditions could not be induced to fly, while beetles reared under long-day conditions would fly occasionally for short periods (less than 3 minutes). The greatest tendency toward flight was by beetles 10-12 days after emerging from diapause, especially female beetles (Mordue and De Kort, 1978). There were a few instances of flights by post-diapause beetles that lasted up to one hour, but generally flights lasted 25-35 minutes. The average flight speed was 40 m/min.

However, Brouwers and De Kort (1979) also studied the flight behavior of Colorado potato beetle. These beetles were 7-15 days old after emerging from the pupae and 7-15 days after diapause-break. This results were in contrast to the observations of Mordue and De Kort (1978). Female post-diapause beetles did not show a greater tendency to fly. But long-day conditioned male beetles which emerged from pupae demonstrated the highest capacity for flight.

In western Massachusetts, the studies by Voss and Ferro (1990 a and b) classified three type of flights of Colorado potato beetle in the field: local flight, long-distance flight, and diapause flight.

Local flight (trivial flight) occurs close to the ground within the host habitat. The functions of this type of flight may be to minimize food source depletion, to find a mate, and to distribute their eggs throughout the host habitat by the females. Overwintered beetles engage this type of flight about 1-2 weeks after colonizing a field, while summer generation beetles engage in this type of flight for about a week before engaging in diapause flight.

Long-distance flight removes beetles from the habitat. The length of long-distance flight can cover up to several kilometers. Under optimal weather conditions (high-temperature, clear skies, and no wind), Colorado potato beetle can fly more than 100 km (Hurst, 1969; Wiktelius, 1981). A possible function of long-distance flight by overwintered beetles maybe to search for host plant and to distribute their eggs in host habitat; this type of flight is rarely observed by summer generation beetles.

Diapause flight takes the beetle from its summer habitat to an uncultured area adjacent to potato field; these sites maybe defined as a wooded area. Once it reaches these sites, it burrows into the soil to diapause. Diapausing Colorado potato beetle densely aggregate in a narrow zone along the forest border (Weber, 1992).

The different generations of beetles engage in different types of flight with respect to colonization, local dispersal and migration.

Post-diapause movement in the spring (May through early June in western Massachusetts) by newly emerged overwintered beetles begins by walking in search of host plants (Voss and Ferro, 1990a). If beetles initially find host plants, they feed and females begin ovipositing about 5-7 days later (Peferoen *et al.*, 1981). If overwintered beetles do not find host plants, they undertake a long-distance flight (Ferro *et al.*, 1991). However, Voss and Ferro (1990b) observed that many post-diapause beetles took off on long-distance flight although food sources were nearby after they had oviposited for several weeks.

Using a computer-linked flight mill system, Ferro *et al.* (1991) showed that overwintered beetles were capable of flying on the first day after emerging. Also they found overwintered unfed beetles flew more often, for longer periods, and for greater distances than fed beetles.

Summer first generation adults feed for 7-9 days to complete development of flight muscle (De Kort, 1969) and then lay a average of 43 eggs (Ferro unpublished data). Under conditions of high temperature and sunshine, some beetles engage in long-distance flight (Caprio and

Grafius, 1990). Following diapause switching (early August western Massachusetts), Voss and Ferro (1990b) observed that many late emerged summer first generation beetles that have fully developed flight muscles also engaged in diapause flight.

The results from flight mill tests showed that unfed summer generation beetles did not fly at all, but fed beetles did (Weber, 1992). However, their flight time was shorter than that of overwintered beetles (Ferro *et al.*, 1991).

Movement by second summer generation beetles that usually emerge in mid-August is almost exclusively by walking (Voss *et al.*, 1988). The short photoperiod and inferior quality food lead to induction of diapause, and flight muscles of these beetles do not fully develop. These beetles diapause in the crop habitat or walk to overwintering site to diapause (Voss and Ferro, 1990 b).

There were two periods of flight activity concurrent with the appearance of post-diapause and first-generation adults in the field, and three periods of walking activity concurrent with post-diapause, first-generation, and second-generation adult beetles in the field (Voss *et al.*1988; Voss and Ferro 1990b). In western Massachusetts, some populations of Colorado potato beetle have two generations each year, while some populations only have one generation each year. Beetles which emerge

just before the inductional diapause switch (first summer generation) have the ability to fly to overwintering sites. However, most of the second generation beetles emerge after the diapause switch (Voss *et al.*, 1988), and these beetles diapause in the potato field or walk to overwintering sites adjacent to the field.

The Development of Flight Muscles

Under long-day conditions (same as most first summer generation), beetles take 7-9 days to complete flight muscle development after emerging from the soil (De Kort, 1969). High quality food is necessary for this process. Without food, flight muscles do not develop and the exoskeleton does not harden, and most beetles die within 10 days (personal observation).

Under short day conditions (same as the second summer generation), the flight muscles of beetles develop to some extent, but the diameter of flight muscle fibrils never reaches the maximal value of those beetles that emerge before the induction of diapause (De Kort, 1969). The flight muscles of diapausing beetles totally degenerate (De Kort, 1969).

The flight muscles of post-diapause beetle regenerate in the spring (Stegwee, 1963; Stegwee, 1964;

De Kort, 1969). Stegwee (1964) studied the respiration of flight muscle sarcosomes and made electron micrographs of longitudinal sections of the dorsoventral flight muscles of newly emerged one day old beetles. The results revealed that complete regeneration had occurred at the time of emergence from the soil. However, De Kort (1969) studied the enzyme activities in the flight muscles of post-diapause beetles and suggested that post-diapause flight muscle development was similar to newly emerged summer generation beetles, i.e. post-diapause beetles still need 7-9 days to develop their flight muscles after emerging from soil. Ferro *et al.* (1991) showed that post-diapause beetles could fly on the flight mill on the first day after emerging. These results support the findings by Stegwee (1964).

De Kort (1969) identified the role of juvenile hormone (JH) in the degeneration and regeneration of flight muscles in the Colorado potato beetle. He used summer generation beetles to establish a positive correlation between the JH levels and the development of flight muscles. Usually, short days induce a decrease in JH titers, movement to diapause sites onset of burrowing behavior, and flight muscle degeneration (De Kort, 1969). Long days result in an increase in JH titers, emergence from the soil, flight muscle regeneration, and movement from diapause site (De Kort, 1969).

Lefevere *et al.* (1989) studied JH metabolism during and after diapause in the Colorado potato beetle. The JH titer remained at a constant low level during diapause. Shortly before emergence from the soil, a slight increase in the rate of JH synthesis occurred in relation to an increase in temperature. The corpora allata (CA) became completely activated only after emergence.

Energy Sources for Flight

The pioneering work of De Kort *et al.* (1973), Mordue *et al.* (1978), Weeda *et al.* (1979), and Brouwers *et al.* (1979) established that the energy sources used by Colorado potato beetle in flight are proline and carbohydrates.

Proline is thought to be the major energy source for Colorado potato beetle flight (Weeda *et al.*, 1979; Brouwers *et al.*, 1979). In long-day conditioned beetles, during the first several minutes of flight, the proline content fell rapidly, while the alanine concentration increased, after 9 minutes of flight, the proline and alanine contents stabilized with little change (Brouwers *et al.*, 1979). Evidence of proline synthesis in the fat body of Colorado potato beetle (Weeda, 1980) suggested that the fat body was the source of proline used during

flight. Alanine was produced by the catabolism of proline in the flight muscles, and then was converted back to proline in the fat body using acetyl CoA, derived from the hydrolysis of stored triacylglycerols (Fig. 1.1).

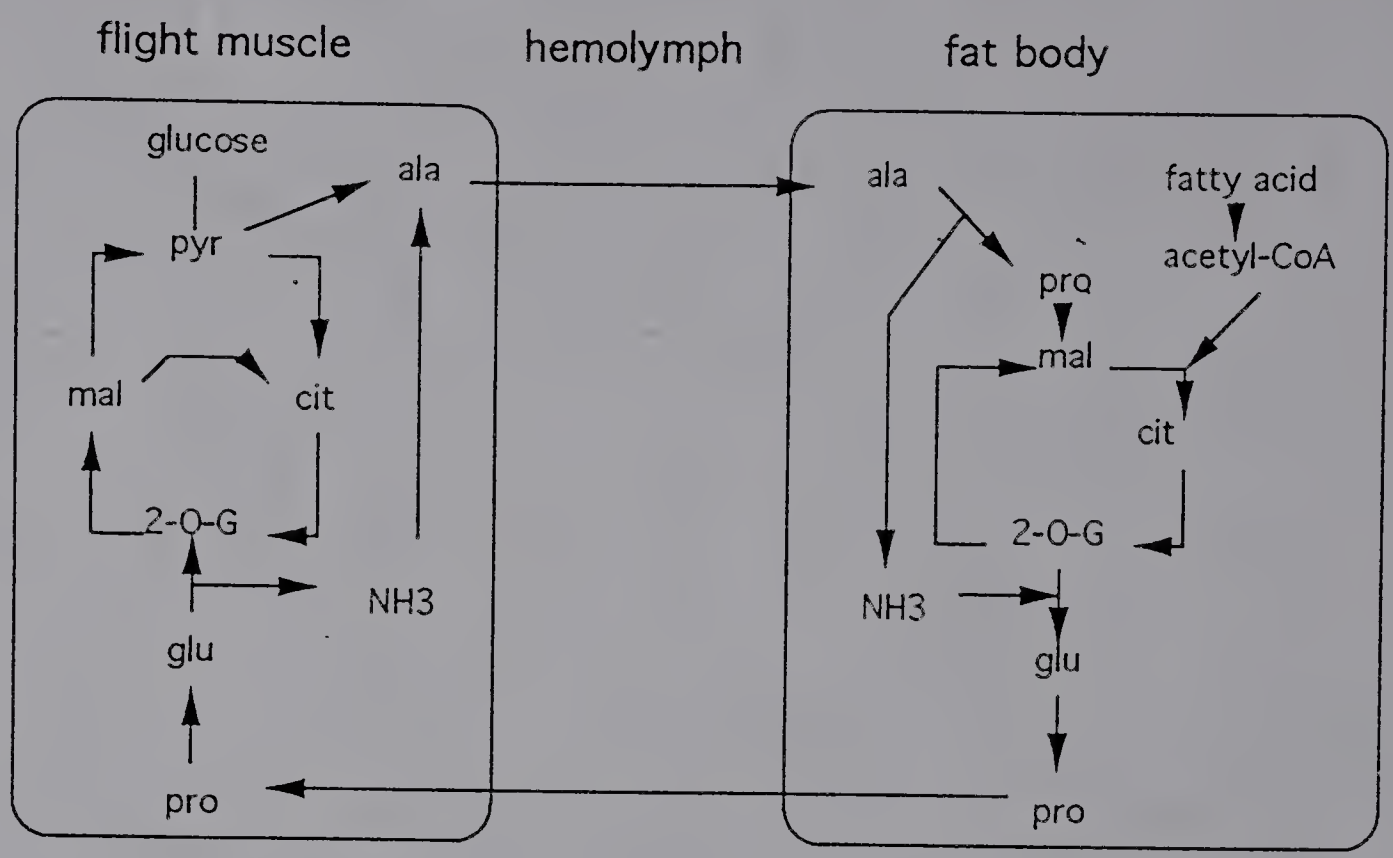


Fig. 1.1. Schematic representation of the pathways of proline oxidation in flight muscle, and proline synthesis in fat body of the Colorado potato beetle (adapted from Bursell, 1981 and Weeda *et al.*, 1980).

Weeda (1980b) showed that the overall reaction was:

$$\text{Alanine} + \text{Acetyl-CoA} + \text{NADH} + H^{+} \rightarrow \text{Proline} + H_2O + NAD^{+} + \text{CoAH}$$

The overall reaction for the partial oxidation of proline to alanine is (Bursell, 1981):

$$\text{Proline} + 2.5O_2 + 14\text{ADP} \longrightarrow \text{Alanine} + H_2O + 2CO_2 + 14\text{ATP}$$

The details of metabolism of proline in the flight muscles are showed in Fig. 1.2. The five-carbon substrate proline is converted to the three-carbon alanine and two remaining carbons appear as CO_2. Reoxidation of reduced coenzymes via the electron transport system allows for phosphorylation of approximately 14 ADP to ATP.

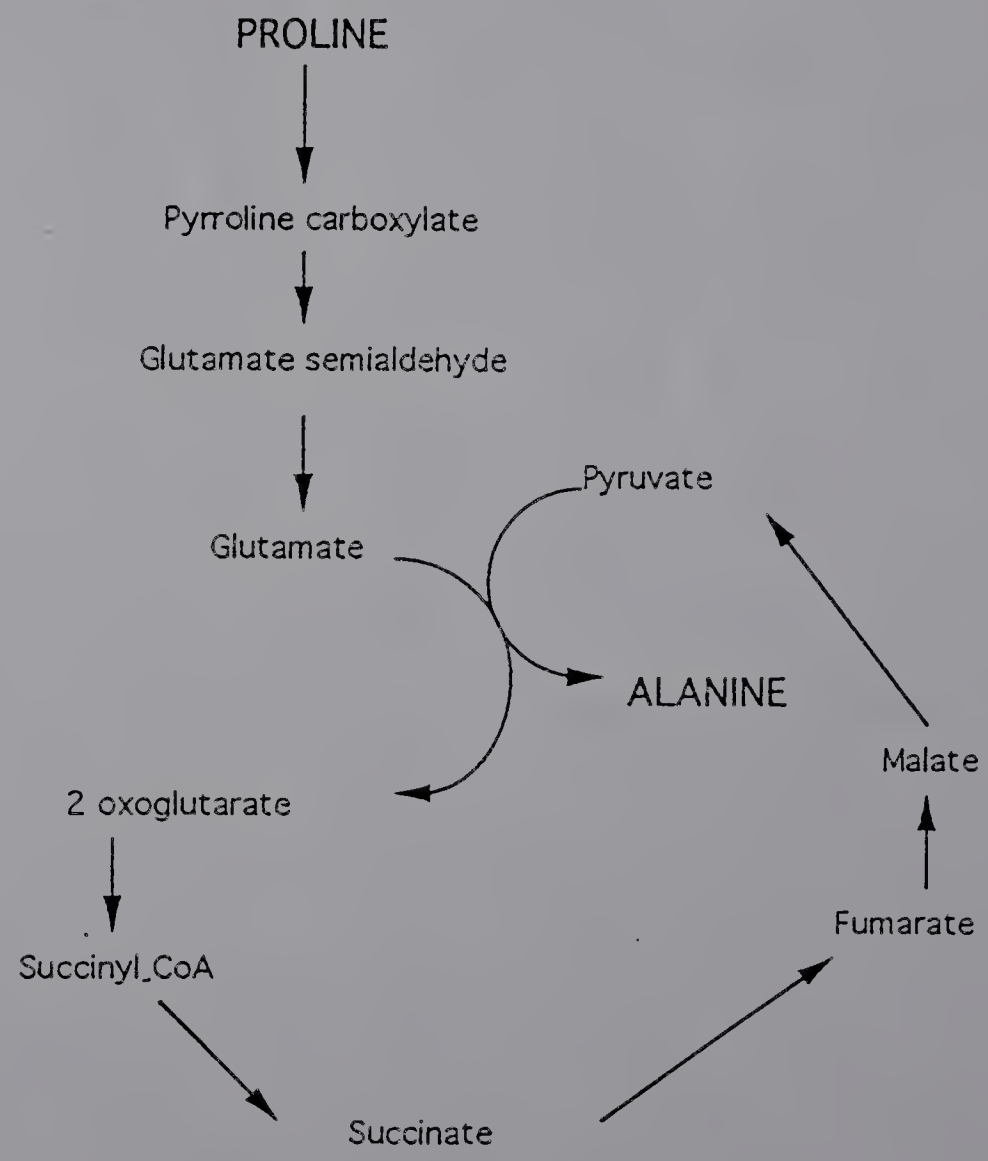


Fig. 1.2. Metabolism of proline in flight muscles of the Colorado potato beetle (adapted from Candy, 1989).

Flight muscles of long-day conditioned Colorado potato beetle have the capacity to utilize carbohydrates during flight (Kahn *et al.*, 1978; Weeda, 1981). Studies by Kahn *et al.* (1978) using long-day conditioned beetles showed that the flight muscle of Colorado potato beetle contained some glycolytic enzymes, although the enzymes were only about half as active as those in the locust. Weeda *et al.* (1979) found that the total carbohydrate, glucose and total glycogen in the flight muscles significantly decreased during flight. Weeda (1981) supported the conclusion that carbohydrates such as glucose also serve as energy sources during flight in the Colorado potato beetle.

In locusts, starvation evokes a decrease of carbohydrate levels and lipid levels in hemolymph, similar metabolic changes occur during flight (Houben, 1976; Goldsworthy *et al.*, 1978). Weeda *et al.* (1979) studied energy sources of long-day conditioned Colorado potato male beetle which had been provided food and water for eight days after emerging from pupa, and then had been deprived of food for a period of 5 days, but had access to water. During the first 4 days of starvation, the glucose concentration in flight muscles and the hemolymph decreased, and proline concentration in the hemolymph and flight muscles remained at a high level. The alanine concentration was lower in the hemolymph and

the fat body than at the onset of starvation. The total lipid levels in hemolymph did not change during this period. However, after four days of starvation, the proline level tended to decrease, while the alanine concentrations showed a sharp rise. The total lipid contents also decreased on the fifth day of starvation.

Metabolic Expenditure During Flight

Energy expenditure of flying insects can be assessed either from the rate of respiratory exchange or from the depletion of the insects' fuel reserve. Usually, the metabolic rates of flying insects are 20 to 100 times more than those of resting insects (Kammer *et al.*, 1978). In the Colorado potato beetle, rates of proline oxidation during flight can be as high as 60 ug/min (Brouwers *et al.*, 1979). However, this was measured under conditions in which gliding flight was not possible, and thus estimates of the metabolic costs of long-distance flight may be too high.

Endocrine Control Of CPB Flight

Juvenile hormone (JH) and adipokinetic hormone (AKH) are the major hormones controlling flight muscle development, flight behavior, and the fuel supply in insects (De Kort, 1969; Rankin, 1989; Candy, 1989).

Johnson (1966) suggested that insect migration was induced by a lack of JH. Johnson (1969) and Rankin (1979) suggested that Colorado potato beetle migration was controlled by JH levels. De Wilde *et al.*(1968) first measured the JH levels in the hemolymph of adult Colorado potato beetle during different stages and under varying conditions. In long-day conditioned beetles, there was a rapid increase in the JH titer at emergence from the soil. In short-day conditioned beetles, JH titer increased initially to intermediate levels (comparing with that in the long-day conditioned beetles), but then rapidly dropped off, preceding the onset of diapause. In post-diapause beetles, JH levels increased rapidly after emergence from the soil. Comparing Colorado potato beetle flight behavior in the field with the changes of JH titer, Voss (1989) suggested that both the levels and the phase of JH activity might control the range of flight behavior in the Colorado potato beetle. Under long-day conditions (July), some newly-emerged adults sustained seasonal nondiapause-mediated migration in

response to increasing, intermediate JH levels; reproduction followed at higher levels of JH (Voss, 1989). As daylengths decreased, newly-emerged adults responded to initially increasing JH levels with diapause flight to overwintering sites, but the JH titer never became sufficient to induce reproduction and beetles entered diapause as the JH titer declined (Johnson, 1969).

The adipokinetic hormones(AKH)/red pigment concentrating hormones (RPCH) are small peptides which are isolated from corpora cardiaca (CC) of insects. The AKH/RPCH family contains structurally related neuropeptides with diverse biological activities. This family derives its name from the first insect neuropeptide to be sequenced and synthesized, the adipokinetic hormone I (AKHI) of locusts, which is similar to the red-pigment concentrating hormone (RPCH) of prawns. These peptides are involved in lipid (adipokinetic hormones) and carbohydrate (hypertrehalosemic hormones) release and activate fat body glycogen phosphorylase. Up to now, the adipokinetic and hypertrehalosemic activities were found in at least 54 insect species of 10 different orders (Gäde, 1990). More than 14 peptides were sequenced from 24 insect species (Gäde, 1990). This peptide family is one of the largest known in nature.

Weeda (1981) showed that a hormone factor like adipokinetic hormone from the Colorado potato beetle CC could stimulate proline synthesis in the fat body, and increase glucose concentration in the hemolymph. Proline synthesis is controlled by feedback inhibition so that the production of proline via the fat body in *vitro* is inhibited by normal concentrations of proline in the hemolymph; but inhibition is relieved when the proline concentration decreases (Weeda, 1981). When CC extracts were injected into the hemolymph, changes in proline concentration appeared to be difficult to detect. This could be the result of feedback inhibition or a consequence of the formation the small amount of proline, which did not significantly alter the concentration in the hemolymph. However, the injection of CC extracts could decrease the amount of alanine in the hemolymph, and this was indicative of proline synthesis. A clearer indication of proline synthesis could be obtained from fat body incubations *in vitro*. The Colorado potato beetle CC extracts, American cockroach CC extracts, locust CC extracts and synthetic AKH-I, all elicit considerable proline synthesis in the Colorado potato beetle fat bodies. The hormone factor in the CC extracts was believed to be a member of the AHK/RPCH family.

Gäde and Kellner (1989) sequenced two eight-amino acid peptides from the Colorado potato beetle CC

extracts. These peptides identified from the Colorado potato beetle are exactly the same as those found in the American cockroach, but their function appears different for these two species (Steele, 1961; 1963; Downer 1972; Weeda, 1981; Gäde and Kellner, 1989). In cockroach, these two peptides stimulate trehalose release into hemolymph, while in the Colorado potato beetle the major functions of these two peptides are to stimulate the synthesis of proline and the release of glucose.

Goldworthy (1979) showed that preflight injection of locust CC extracts into locust not only elevated hemolymph diacylglycerol levels, but also increased flight speed. However, the injection of locust CC extracts did not stimulate the locust to engage in flight. The relationship between these hormonal factors and flight in the Colorado potato beetle remains unclear.

The hormones in the AKH/RPCH family can control the fuel supply system at many different points. They can affect substrate storage, mobilization, transport, uptake, and utilization (Rankin, 1989). Unfortunately, few detailed studies of these have been undertaken in the Colorado potato beetle.

CHAPTER 2

FUEL UTILIZATION OF FLIGHT IN OVERWINTERED AND SUMMER GENERATION COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY) (COLEOPTERA:CHRYSOMELIDAE)

Introduction

Fuel sources for flight by the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) were first studied by De Kort in 1973. Since then the amino acid proline and the carbohydrate glucose were found to be energy sources for flight by Colorado potato beetles (Khanand De Kort, 1978; Mordue and De Kort, 1978; Brouwers and De Kort, 1979; Weeda *et al.*, 1980a; Weeda *et al.*, 1980b).

Most of these experiments were done on the 7-12 day old adult beetles which were reared under a long day photoperiod (>14 hour light) as described by De Kort (1969). For these beetles, proline and total carbohydrate concentrations in the hemolymph and flight muscles significantly decreased during 9 to 20 minute

flight, but the lipid levels did not change (Brouwers *et al.*, 1979; Weeda *et al.*, 1979).

However, for 10-12 days after diapause in female beetles, Mordueand De Kort (1978) found that the proline concentration in the hemolymph significantly decreased after 20 minutes of flight, also the total lipid concentration levels in the hemolymph increased during the same flight period, but the concentration of total carbohydrate did not change significantly.

Recently, research on the biology and behavior of flight by beetles observed in the field has been elucidated. Voss and Ferro (1990 a and b) showed the overwintered generation and summer generation Colorado potato beetle have different flight patterns in western Massachusetts. Ferro *et al.*(1991) compared flight abilities between the starved and fed overwintered beetles and found starved overwintered females flew almost four times as much as fed overwintered females. Weber (1992) showed that the flight abilities between post-diapause and summer generation beetles were different and the beetles which fed on different diets had variable flight patterns.

The objective of this work was to try to compare fuel utilization for flight in post-diapause and summer generation beetles under different nutritional conditions.

Materials and Methods

Post-diapause (overwintered) Colorado potato beetle adults were collected from a potato field in South Deerfield, Massachusetts. Some active beetles (the second summer generation and the late emerged first generation) were collected in late August, 1992. These beetles were provided potato foliage in cages under natural conditions (short-day conditions, <14 hour photo period) until the beetles went into diapause. Some diapausing beetles were collected from soil in early April of 1993 before soil temperatures reached 8°C. All of these beetles were sterilized with a 5% bleach solution, then covered with 10 ml of pasteurized potting soil, and were kept in a cold room (4°C). After three months of diapause development, beetles were shifted to a growth chamber maintained at 27°C, 75% RH, and a photoperiod of 16:8(L:D). After emerging from the soil, beetles were either fed or left unfed and maintained at 27°C and a long-day condition (16:8, L:D). Fed beetles were provided fresh potato foliage and water, and unfed beetles were provided water only. Nine to 11 days after emergence, the fed and unfed female beetles were tethered on the flight mill and allowed to fly.

First generation (summer generation) beetles were reared from larva to adult in a green house at 25-27°C,

and a 16:8 (L:D) photoperiod. When the adults emerged from the soil, female beetles were reared in a growth chamber at 27°C, 75% RH, and a 16:8 (L:D) photoperiod. These beetles were provided fresh potato foliage and water daily. After 9-11 days, beetles were place on the flight mill to allow them to fly.

Flight mill. The flight mill system used in our experiments consisted of five components: the flight mills, custom external and internal interface boards, computer hardware, custom software and mill support structure with lighting. The details of flight mill structure and operation were described by Weber *et al.* (1993). The temperature in the flight mill room was maintained between 25°C to 27°C, and 65%-70% RH, and air condition was on low fan. Mixed-spectrum incandescent overhead lighting totaling 1.05 KW was wired through a rheostat for lighting adjustment, with light intensity of maximum output of 1912 lux.

Hemolymph sampling. After beetles flew for one minute, two minutes, four minutes, 10 minutes, 25 minutes, and 60 minutes, the right fore and hind wings were removed. Hemolymph was extracted from beetle by placing a micropipet on the beetle's wounded wing base within 15 seconds after flight was terminated. Usually, 10-25 ul hemolymph could be extracted from one beetle,

and this amount was enough for the determination of proline, alanine, total lipids, and total carbohydrates. Crystals of phenylthiourea were added to the hemolymph to prevent tyrosinase activity. The hemolymph samples were stored at -20°C until analyzed. For analyses, the blood samples were stirred and centrifuged at 1200g for five minutes, and these cell-free hemolymph samples were used.

Total lipid determination. Total lipid concentrations in the hemolymph were determined by of the method described by Goldsworthy *et al.*(1972). This method used the reaction with vanillin-phosphoric acid reagent and concentrated sulfuric acid. In the present study, some modifications were made. One ul hemolymph was mixed with 100 ul of concentrated sulfuric acid. The mixture was heated in a boiling water bath for 10 minutes. After cooling, 400 ul of the vanillin solution (13 mM vanillin in 11.8 M phosphoric acid) was added to each tube. After 30 minutes, the optical density of the solution was determined at 546 nm. Commercial vegetable oil (pure soy bean oil) was used as the lipid standard (Van Handel, 1985). The concentrations of 0, 2, 4, 6, 8, and 10 ug/ul standard lipid solutions were made by dissolving vegetable oil in chloroform. A typical standard curve for the total lipid determination is shown in Fig. 2.1.

Total carbohydrate determination. Total carbohydrate concentrations were determined using the method described by Dubois *et al.* (1956). In this method, phenol was utilized as the specific organic color-developing agent. In addition to this method being simple and sensitive, it was largely unaffected by the presence of protein. In the present study, one ul of cell-free hemolymph was dissolved in 199 ul distilled water and mixed with five ul of the phenol reagent (80% by weight, prepared by adding 2 g of distilled water to 8 g of redistilled, reagent grade phenol). Then 500 ul of concentrated sulfuric acid was rapidly added. After the samples were held at room temperature for 30 minutes, the color remained stable for several hours. During this time, the optical density was determined at 485 nm. All the samples were assayed in triplicate. Pure D-glucose crystal was dissolved in the distilled water as the standard. A typical standard curve for the total carbohydrate determintion is displayed in Fig. 2.2.

Since the heat required for color development was provided by the exothermic reaction of sulfuric acid and water, it was desirable to add the acid rapidly and directly onto the surface of the sample solution. It was very important that these all tubes in a given experiment be of the same size and thickness to reduce experimental variations. Borex test tubes (12 x 75 mm) were used to

permit good mixing and the same dissipation of heat in all directions.

Proline determination. Proline concentrations in the hemolymph were measured using the method described by Bergman and Loxley (1970). One ul of cell-free hemolymph was dissolved in 99 ul of 0.3 N hydrochloric acid. Freshly prepared 1.25 M sodium nitrite solution (20 ul) was added to each tube, contents were mixed, and allowed to stand for 20 minutes at room temperature. Twenty ul of 1.25 M ammonium chloride solution were then added to each tube, the contents were mixed again, and 100 ul of concentrated hydrochloric acid were added. The contents of the tubes were mixed and then heated for 20 minutes in a boiling water bath. After the tubes were cooled, 100 ul of 10 N sodium hydroxide solution were added. In these procedures, nitrous acid was produced to eliminate the interference from other amino acids.

Aliquots of these solutions (300 ul) after the nitrous acid treatment were pipetted into 5-ml test tubes. Three hundred ul of a phosphate buffer solution (5.32 M in phosphoric acid and 3.88 M in sodium dihydrogen phosphate) and 600 ul ninhydrin solution (freshly prepared 3% aqueous solution of ninhydrin, indanetrione monohydrate) were added into each tube. The contents of the tubes were mixed and the tubes were put into a boiling water bath. After 100 minutes of boiling,

the red reaction product was stable indefinitely. When the tubes were cooled, 2 ml of glacial acetic acid were added to each tube. The absorbances of the resulting solutions were measured at a wavelength of 512 nm within 4 hours of the reaction.

Pure proline crystals were used for the standard curve. The concentrations of 0, 2.5, 5, 10, and 20 ug/ul proline solutions were made by diluting with 0.3 N hydrochloric acid. All the procedures for the standard curve were the same as those for the samples. A typical standard curve for proline is shown in Fig. 2.3.

Alanine determination. Alanine concentrations were determined following the basic method of Willamson (1970) using alanine dehydrogenase. L-alanine was oxidized to pyruvate and ammonia in the presence of NAD^+ and alanine dehydrogenase.

$$\text{L-Alanine} + NAD^+ + H_2O \longrightarrow \text{Pyruvate} + NADH + NH_4^+$$

Hemolymph was deproteinized with methanol instead of perchloric acid. Two ul of cell-free hemolymph were dissolved in 60 ul of 60% methanol solution in a 1.5 ml plastic tube. The contents of tube were mixed and centrifuged at 800g for 10 minutes. The supernatant was transferred to one clear tube, and the solution was dried under a gentle stream of air. Five hundred ul of distilled water were added into the tube and mixed.

After these deproteinized procedures, 250 ul of sample solution were pipetted into a 0.5 ml cuvette. Then 250 ul of hydrazine/Tris buffer (Tris, 40 mmol/l; hydrazine, 1 mol/l; EDTA, 1.4 mmol/l; pH 9.0) were added into the cuvette. Twenty-five ul NAD^+ solution (β-NAD, 24 mmol/l) were added. The contents of the cuvette were mixed, and absorbance A_1 was read at the wavelength 365 nm. 2.5 ul alanine dehydrogenase (150 kU/l) were pipetted into the cuvette and mixed. The absorbance was measured at 40, 50, and 60 minutes to determine the final absorbance A_2. The alanine concentration was calculated by using ΔA (A_2 - A_1) in standard curve equation. Pure alanine was used to make the standard curve. Bovine serum albumin (BSA) was added to pure alanine solutions to make the final protein concentration of 10 mg/ml (about equal to the protein concentration in the hemolymph of Colorado potato beetles). The standard alanine solutions were deproteinized with methanol (final concentration was about 60%). A standard curve was established with each alanine determination. A typical standard curve for alanine is presented in Fig. 2.4.

Results

Age-related changes of metabolite concentrations in hemolymph

Proline is a major free amino acid in CPB hemolymph, and is always found at higher concentrations than other amino acids (De Kort and Kramer, 1976). The proline concentration changes in the hemolymph of overwintered fed and unfed as well as summer generation beetles are presented in Fig. 2.5, Fig. 2.6, and Fig. 2.7.

For post-diapause (overwintered) beetles, proline concentrations were relatively high (11.2 ug/ul) in hemolymph when beetles just emerged from the soil. The concentrations of proline in overwintered fed beetles increased from 11.1 ug/ul to 13.6 ug/ul within eight days after emerging, then remained at these levels until day 20. While in the unfed beetles, proline concentrations remained at the same levels from day 0 to day 20 (Fig. 2.5, and Fig. 2.6).

For summer generation beetles, proline concentrations were as low as 1.9 ug/ul in the hemolymph for adult females that had just emerged from the soil. The proline concentrations in the summer generation beetles increased gradually from 1.9 ug/ul over 20 days to 14.0 ug/ul. Over the first 8 days, the proline

concentrations rapidly increased to 10.0 ug/ul (Fig. 2.7).

Compared to proline, alanine concentrations were very low for all beetles. Alanine concentrations in the hemolymph of newly emerged overwintered beetles were very low (<0.1 ug/ul), almost undetectable. The change patterns of alanine concentrations looked almost the same for fed and unfed overwintered beetles. For fed beetles, alanine concentrations reached about 0.4 ug/ul at day 8-12. For unfed beetles, alanine concentrations also increased to 0.3 ug/ul by day 8. After day 8, alanine concentrations decreased to about 0.1 ug/ul at day 16-20 in both fed and unfed beetles (Fig. 2.5 and Fig. 2.6).

In summer generation beetles, alanine concentrations were generally higher than those in fed and unfed overwintered beetles. After beetles emerged and began to feed, alanine concentrations gradually increased to 0.6 ug/ul by day 12, and then gradually decreased to 0.1 ug/ul by day 20 (Fig. 2.7).

Total carbohydrate concentrations for overwintered fed beetles were about 7.4 ug/ul for newly emerged beetles and gradually increased to 13.5 ug/ul by day 16 and remained stable through day 20. While, the total carbohydrate concentrations for unfed overwintered

beetles decreased from 7.4 ug/ul to 4.7 ug/ul by day 8, and remained stable through the duration of the experiment (Fig. 2.5 and Fig. 2.6).

The total carbohydrate concentrations for summer generation beetles were 6.3 ug/ul for newly emergied beetles and increased to 11.6 ug/ul by day 12 and peaked at 13.0 at day 20 (Fig. 2.7).

The concentrations of total lipid in newly emerged post-diapause beetles were 5.3 ug/ul. Lipid concentrations in fed beetles increased to 9.1 ug/ul by day 12, and the lipid concentrations for unfed beetles gradually decreased to 1.8 ug/ul on day 12, and remained stable for the duration of the experiment (Fig. 2.5 and Fig. 2.6).

Total lipid concentrations in hemolymph of newly emerged summer generation beetles were only 1.7 ug/ul and rapidly increased to 9.2 ug/ul by day 8. By day 20, the total lipid concentration in hemolymph reached 11.2 ug/ul (Fig. 2.7).

In general, the age-related changes in the concentrations of metabolites in the hemolymph were different between overwintered fed and unfed beetles. Proline, total carbohydrates, and total lipids were significantly higher in overwintered fed beetles than in unfed beetles. The changes in metabolites were also

different for overwintered and summer generation beetles. In overwintered beetles, the concentrations of proline, total carbohydrates, and total lipids increased (fed) or decreased (unfed) in the first 8-12 days, then stabilized at these levels. While these metabolites for summer generation (fed) beetles always increased through the duration of the experiment.

Energy substrate utilization during flight

The proline concentration in the hemolymph for 9-11 day post-diapause fed beetle was about 13.0 ug/ul before flight. There was little deviation from this level over the 60 minute flight period (Fig. 2.8). For 9-11 day unfed post-diapause beetles, there was an initial rapid reduction in the proline concentration after 10 minute flight (Mann-Whitney test significant, $p<0.01$), then the proline concentration slightly increased to a stable level throughout the duration of a 60 minute flight (Fig. 2.9). The proline concentration for the summer generation beetles, which had fed for 9-11 day, was 16.7 ug/ul in the hemolymph at the beginning of the flight and dipped slightly to about 13.0 ug/ul after 4 minutes of flight (Mann-Whitney test significant, $p<0.05$), and kept this proline concentration level for the flight of 60 minutes (Fig. 2.10).

The alanine concentration increased significantly during the first 10 minute of flight from 0.4 ug/ul in

the hemolymph at the beginning to 2.2 ug/ul (Mann-Whitney test significant, $p<0.01$) for fed post-diapause beetles (Fig. 2.8). The alanine concentration for unfed post-diapause beetles was 0.2 ug/ul of the hemolymph at the beginning of flight and increased to 4.9 ug/ul after 10 minute of flight (Mann-Whitney test significant, $p<0.01$) and then stabilized at 4.4 ug/ul for the remainder of the flight period (Fig. 2.9). The alanine concentration for summer generation beetles was 0.3 ug/ul prior to flight and within 2 minutes of flight elevated to 2.0 ug/ul and reached 3.7 ug/ul by 10 minutes (Mann-Whitney test significant, $p<0.01$), and then gradually declined to 2.5 ug/ul by the time of flight was terminated (Fig. 2.10).

The total carbohydrate concentrations in post-diapause fed beetles declined during the first 10 minutes of flight from 13.8 ug/ul to 9.6 ug/ul (Mann-Whitney test significant, $p<0.05$). Then total carbohydrate concentrations gradually increased to about 11.0 ug/ul during a long flight (60 minutes) (Fig. 2.8). The total carbohydrate concentrations in post-diapause unfed beetles fluctuated around 6.0 ug/ul for the first 10 minutes of flight then stabilized at this level for the next 50 minutes of flight (Fig. 2.9). The total carbohydrate concentration for summer generation beetle was 14.6 ug/ul prior to flight and quickly dropped to 12.8 ug/ul within 2 minutes and then remained around 12.0

ug/ul for the flight period (Mann-Whitney test significant, $p<0.05$) (Fig. 2.10).

The total lipid concentration for overwintered fed beetles was 10.5 ug/ul before flight and rapidly increased to 11.9 ug/ul within the first 2 minutes of flight then quickly dropped to 8.5 ug/ul by 4 minutes and gradually declined to 7.1 ug/ul by the time flight was terminated (Mann-Whitney test significant, $p<0.01$) (Fig. 2.8). The total lipid concentration in the hemolymph for post-diapause unfed beetle prior to flight was 4.4 ug/ul and rapidly increased to 6.6 ug/ul within 4 minutes of flight and stabilized at about 5.0 ug/ul for the long time flight (Fig. 2.9). The total lipid concentration in the hemolymph for summer generation beetle fluctuated around 11.0 ug/ul for the entire flight period (Fig. 2.10).

Discussion

Energetic substrates accumulation and utilization

Prior to entering diapause in the fall, beetles must store enough energetic substrates to survive the winter, regenerate flight muscles and disperse to find host after the termination of diapause. In diapausing beetles, the body lipid contents are higher than in non-diapause beetles (De Kort, 1969). After post-diapause beetles emerge from the soil, if they find food, beetles feed

immediately. Within 12-16 days, the concentrations of proline, total lipid, and total carbohydrate in the hemolymph increased quickly and then remained at these levels (Fig. 2.5). The rates of increase were 25% for proline, 82.6% for total carbohydrates, and 56.7% for total lipids (Fig. 2.5). However, if beetles did not find food, total carbohydrate concentrations in the hemolymph decreased 36.7% within 8 days (Fig. 2.6), and total lipid concentrations in the hemolymph decreased 55.1% within 12 days (Fig. 2.6), but the proline concentration in the hemolymph remained stable for the duration of experiment (20 days) (Fig. 2.6). In this present study, I did not determine the contents of lipid, carbohydrates, and amino acids in either flight muscles or fat bodies. However, the metabolite concentrations in the hemolymph may still give some valuable information about energy accumulation in post-diapause fed beetlts and consumption in unfed beetles. The proline concentration seems to be highly homostatic in the hemolymph for both fed and unfed post-diapause beetles. This indicates that proline may play an important role in providing energy to maintain metabolism in beetle bodies. The concentrations of total carbohydrates and total lipids in the hemolymph were highly variable under different nutrition conditions.

When summer generation beetles emerged from the soil, they needed plenty of energy to complete cuticle

and flight muscle development (De Kort, 1969). These beetles had little energy reserves at this time. The concentrations of total lipids, total carbohydrates and proline in hemolymph of newly emerged summer generation beetles were much lower than those of newly emerged post-diapause beetles. If summer generation beetles did not find food, beetles died within 10 days (personal observation). If summer generation beetles found food, the concentrations of proline, total carbohydrates, and total lipids in the hemolymph quickly increased within 8 days. The proline concentration increased 425.4% (Fig. 2.7), total carbohydrates increased 76.9% (Fig. 2.7), and total lipids increased 430.5% (Fig. 2.7). This process of energy accumalation is significantly different from that in post-diapause beetles.

Fuel utilization during flight

Insect flight is very energy-demanding. The most common fuels for insect flight are carbohydrates and lipids and, to a lesser extent, amino acids (Beenakkers, 1984). Insect species are sometimes classified into groups based on the energy source used for flight, some insects strictly use carbohydrates, or lipids, or carbohydrates and lipids, and or amino acids (Beenakkers *et al.*, 1985). In most species of Diptera and Hymenoptera, carbohydrates constitute the predominant substrate for flight, whereas in many species of

Lepidoptera and Orthoptera carbohydrates are utilized in combination with lipids. For example, locusts almost exclusively utilize carbohydrates at the initiation of flight, whereas lipids are the main fuel during sustained flight, when carbohydrates are only a minor contributition to the total energy supply (Beenakkers *et al.*,1981; Steel, 1981). In Lepidoptera and Orthoptera, insects performing long range flight rely heavily on lipids as a fuel for flight muscle metabolism (Beenakkers *et al.*, 1985).

Carbohydrates yield energy easily and can easily be transported in hemolymph, as they are quite soluble in water. However, carbohydrates have the disadvantage that they are osmotically active, and that the energy content per weight unit is small (0.18 mole ATP/g) compared to lipids (Steele, 1981).

Lipids have a much higher energy content (0.65 mole ATP/g) and are osmotically inactive. It is much more economical to store lipids for flight, especially for long flights, and for insects which do not feed for long periods. Lipids have one major drawback; they are insoluble in aqueous media, such as hemolymph. This is especially so for neutral lipids like diacylglycerols which are the form of lipids transported in insects. Thus, insects use special transport molecules, the lipoproteins to transfer lipids in hemolymph (Beenakkers *et al.*, 1981; Chino, 1981).

The amino acid proline is utilized as the major fuel by some insects (Bursell, 1981). Many insects of the advanced orders have a high level of amino acids in hemolymph with a general predominance of proline and glutamate. Proline gives a high energy yield; 14 moles of ATP/mole of proline or, on a weight basis, 0.52 mole ATP/g which is nearly as much as from lipids (Bursell, 1981).

The question has been raised, why Colorado potato beetles use proline as their major flight substrate? Bursell (1981) answered this question: to constitute a suitable flight energy substrate, an amino acid needs to produce a high yield of metabolic energy. Secondly, it should be highly soluble, so that a large amount of energy could be held in solution in body fluids and, once tissue reserves have been depleted, so that steep concentration gradients could be established between hemolymph and flight muscles. Third, this amino acid shoud contain fewer nitrogen, because detoxitication by conversion to uric acid requires a cost of 1.5 mole of ATP per atom of nitrogen. In considering all factors, proline is an ideal energy substrate for flight.

It could still be asked why an amino acid should be used as a flight energy source instead of converting it to carbohydrate, which combines high solubility with reasonable energy yield and in addition has the potential for convenient storage? This is because that the

requirement for disposal of amino nitrogen in the form of uric acid was not easily reconciled with the conversion of nutrient to carbohydrate (Bursell, 1981). The main uric acid precursor, glycine, plus its metabolically close relatives alanine and serine, are the very amino acids which could most easily be converted to 3-carbon intermediates of the gluconeogenic pathway, but nearly 60% of the total intake of these three amino acids is required to dispose, as uric acid, of the protein nitrogen with which they are associated (McCabe and Bursell, 1975).

In Colorado potato beetles, former studies have shown that proline and carbohydrates are fuels for flight in summer generation beetles (De Kort et al., 1973; Khan and De Kort, 1978; Mordue and De Kort, 1978; Weeda, 1979). The results from our experiments clearly support these findings. In this present study, the proline concentrations in the hemolymph for summer generation beetles decreased 22.4% after 4 minutes flight and then stayed stable at this level (Fig. 2.10). And alanine concentration increased 1180.8% in the hemolymph for summer generation beetles after 10 minutes of flight (Fig. 2.10). The total carbohydrate concentration in the hemolymph for summer generation beetles decreased 19.1% after 25 minutes of flight (Fig. 2.10).

For post-diapause unfed beetles, only proline concentration in the hemolymph significantly decreased

when beetles flied (Fig. 2.6), and alanine concentration increased 2441.9% after 10 mintues of flight (Fig. 2.6). Proline seems to be the sole energy source in flight for post-diapause unfed beetles. While for post-diapause fed beetles, proline concentration in the hemolymph did not significantly decrease when beetles flew (Fig. 2.8), however, the alanine concentration increased 466.3% after 10 minutes of flight (Fig. 2.8). This indicates that proline still was the energy source for flight in post-diapause fed beetles. The total carbohydrate and total lipid concentrations in the hemolymph for post-diapause fed beetles also significantly decreased during flight (Fig. 2.5). These suggested that the carbohydrates and lipids might contriubte to energy supplies for flight in post-diapause fed beetles. This conclusion is also supported by the fact that alanine concentration only increased 466.3% (up to 2.2 ug/ul) after 10 minutes of flight, compared to an increase of 2441.9% (up to 4.9 ug/ul) after 10 minutes of flight for post-diapause unfed beetles. Carbohydrates seem to be a reasonable energy source for flight in post-diapause fed beetles. However, it is surprising that lipids also played a role in energy supplies of flight for fed post-diapause beetles, and this might need further confirmation.

In this study, the measurements of metabolite concentration changes during flight indicated that the concentrations of total carbohydrates and total lipids

always jumped to higher levels in the first few minutes of flight then dropped back (Fig 2.8, Fig 2.9, and unpublished data). These jumps had no statistical differences, but happened very often so that it cannot be discounted as variations.

Proline and alanine work as energy shuttles to transfer 2 carbon acetyl-CoA from fat body to flight muscles through the heomlymph (Bursell, 1981). My studies indicate that the increase in alanine concentration can work as a more sensitive index for oxidation of proline. However, it is difficult to estimate the percentages in the combinative utilization of proline and carbohydrates in flight by the measurement of the increase and decrease of metabolite concentrations which are present in the hemolymph during flight.

For many insect species, trehalose is the major carbohydrate in the hemolymph. In locust and blowfly, hemolymph trehalose is the enery source during the first 20-30 min of flight (Candy, 1989). However, in Colorado potato beetle, glucose was reported to be used in the flight of summer generation beetles (Weeda *et al.*, 1979, 1980). I only determinated the concentration changes of total carbohydrate in the hemolymph during flight. Which kind of carbohydrates are used in post-diapause fed beetles remains unclear.

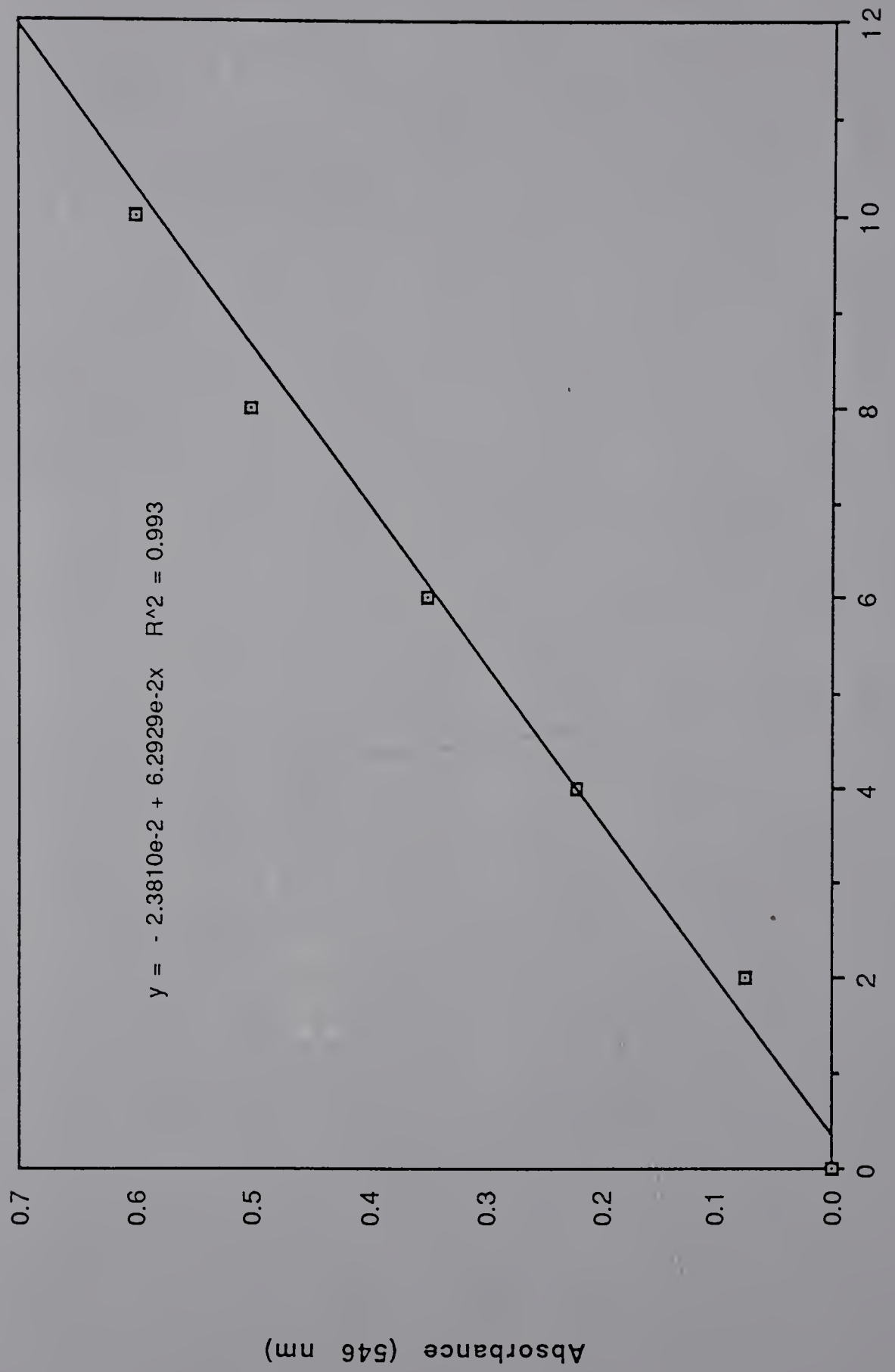


Fig. 2.1. Standard curve for total lipids

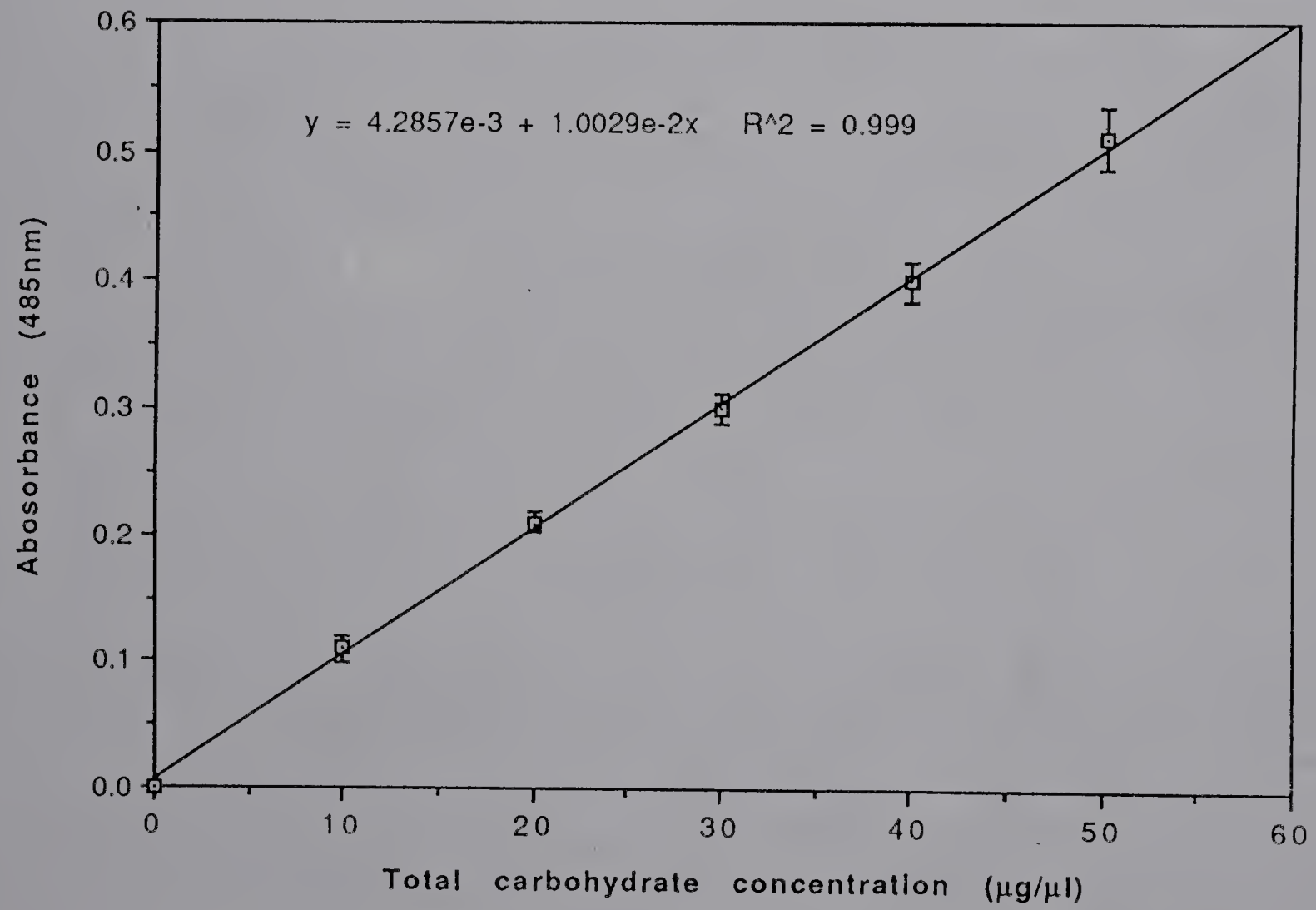


Fig. 2.2. Standard curve for total carbohydrates

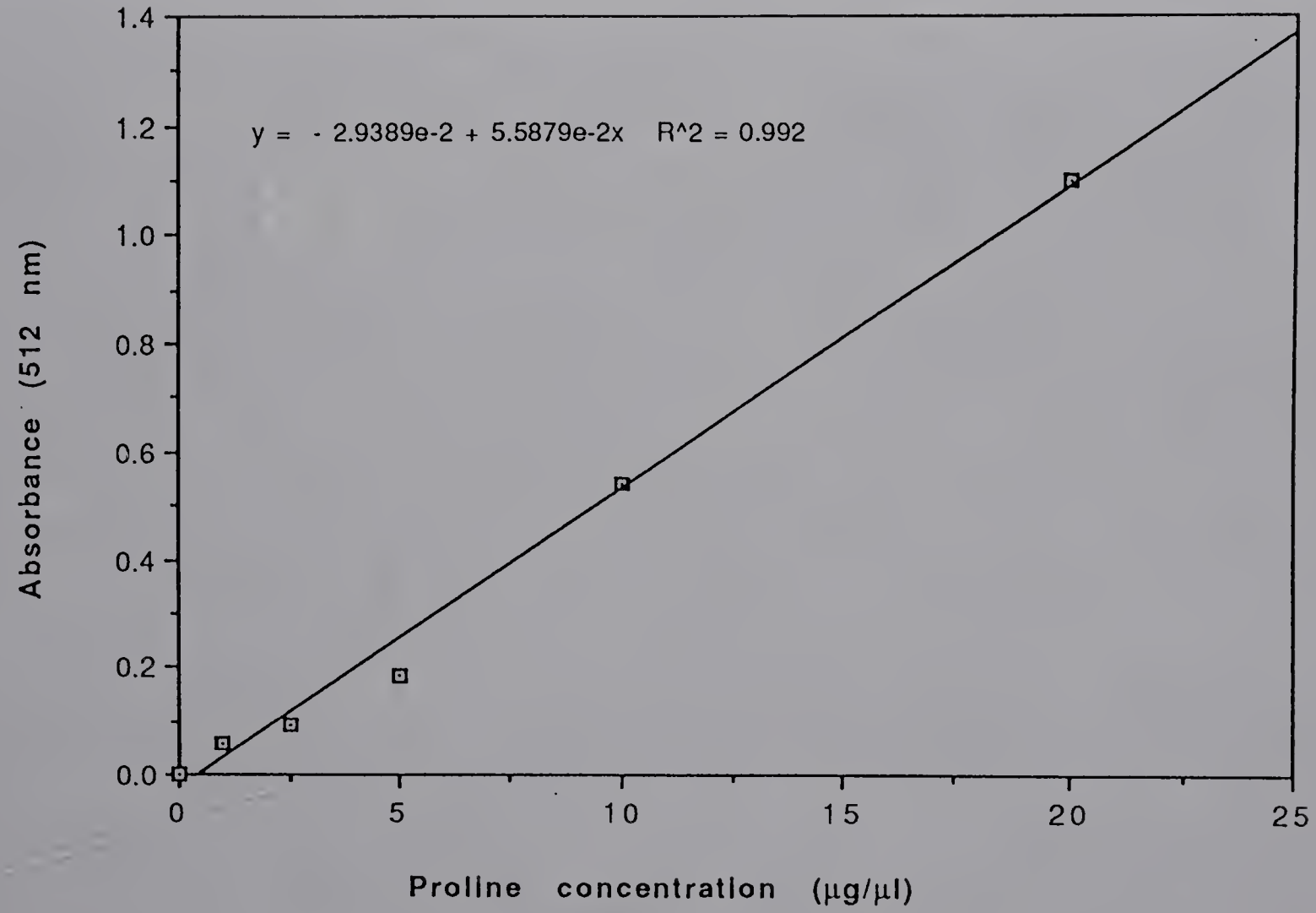


Fig. 2.3. Standard curve for proline

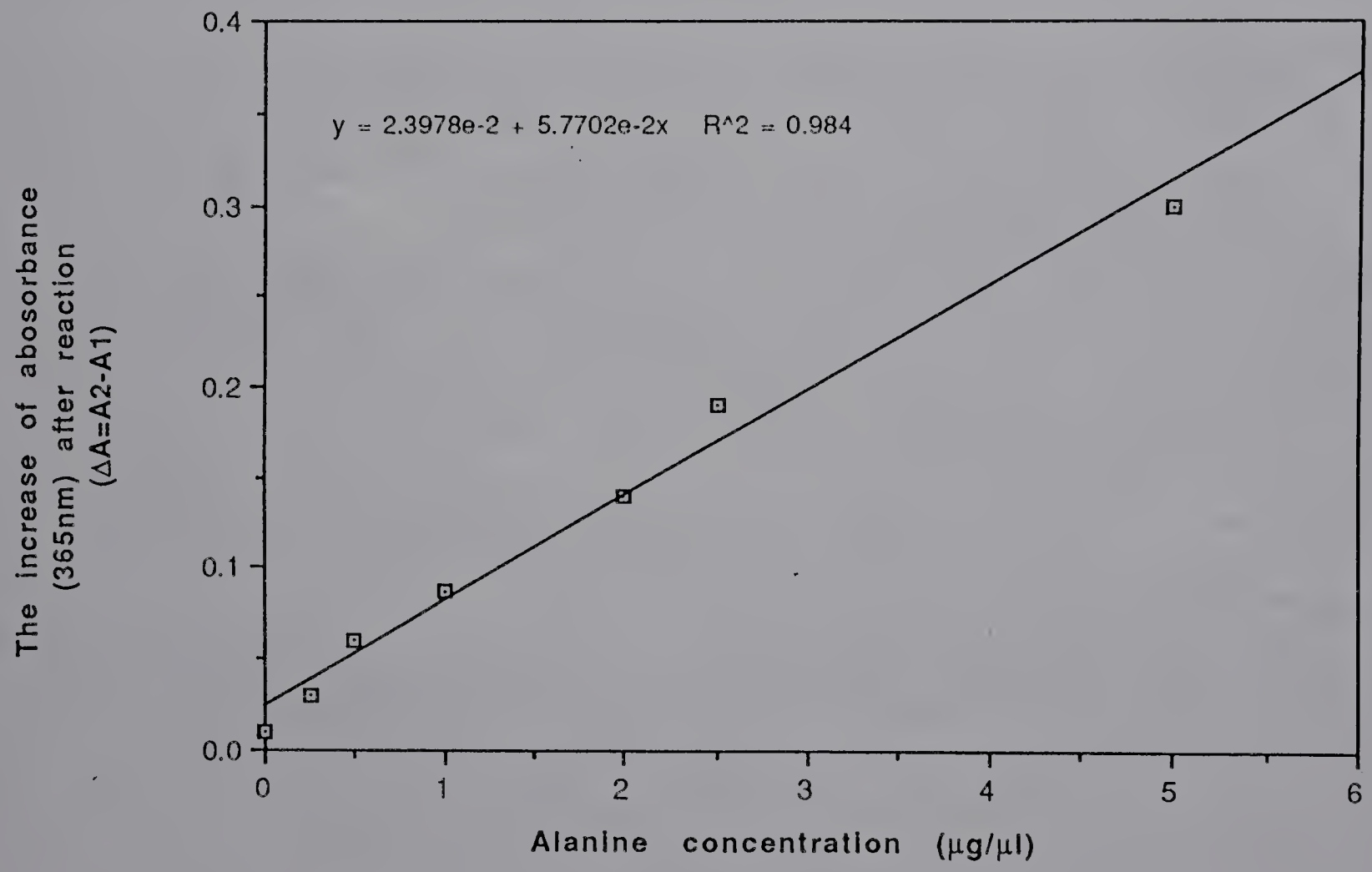


Fig. 2.4. Standard curve for alanine

Fig. 2.5. The concentration changes of proline, alanine, total carbohydrates, and total lipids in the hemolymph for post-diapause fed female beetles.

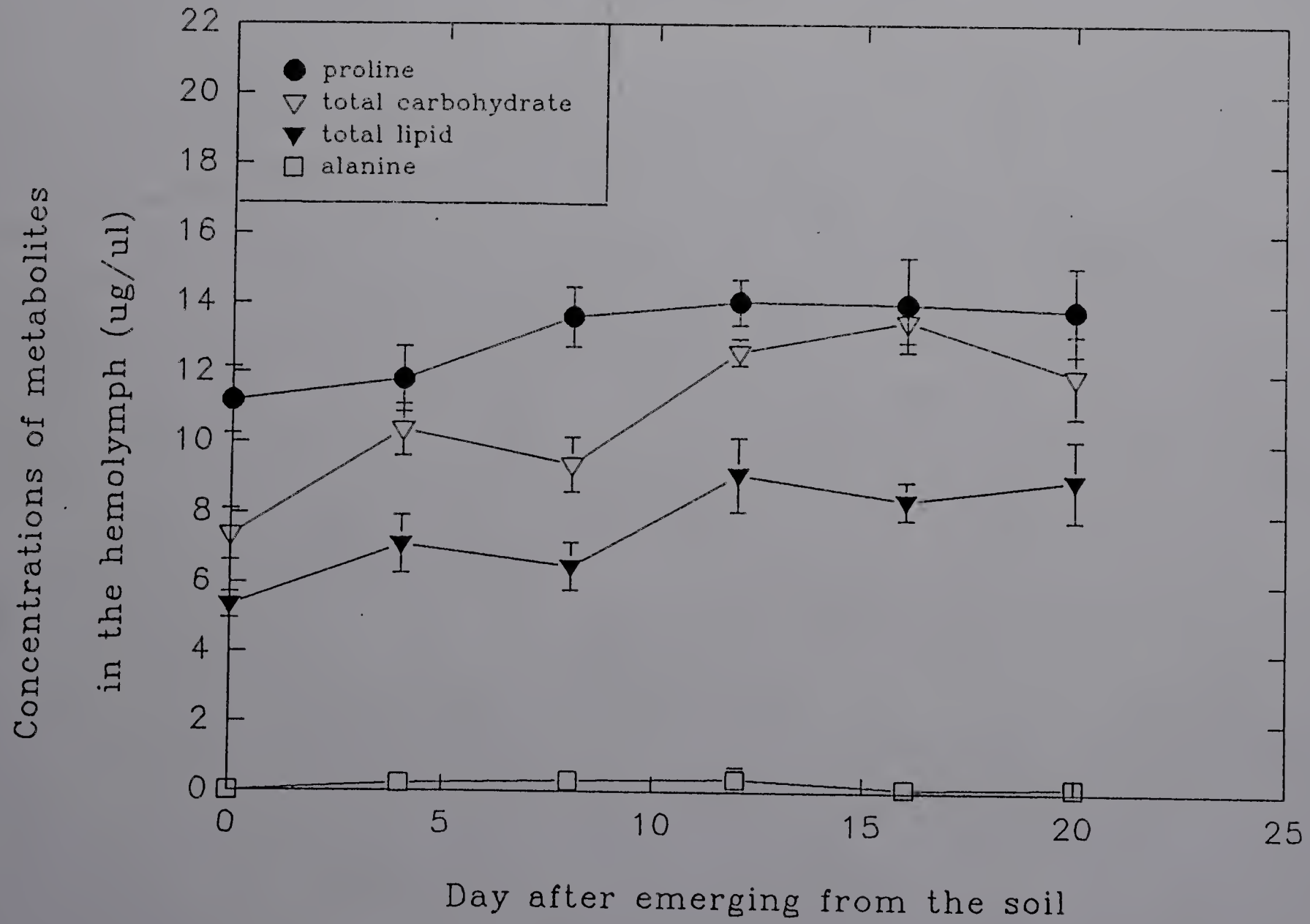
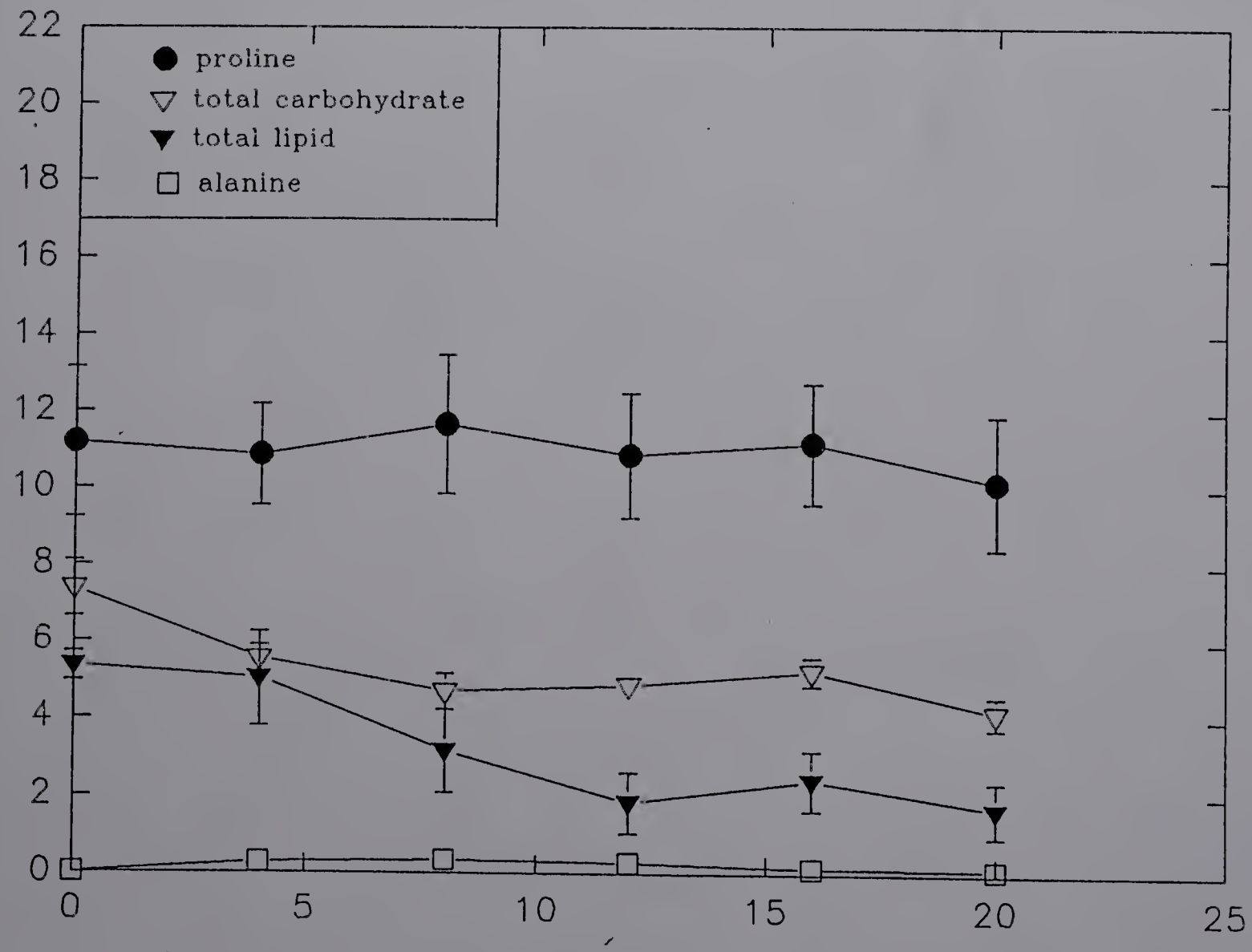

proline
total carbohydrate
total lipid
alanine
Concentrations of metabolites in the hemolymph (ug/ul)
Day after emerging from the soil
0
2
4
6
8
10
12
14
16
18
20
22
0
5
10
15
20
25

Fig. 2.6. The concentration changes of proline, alanine, total carbohydrates, and total lipids in the hemolymph for post-diapause unfed female beetles.

The concentrations of metabolites in the hemolymph (ug/ul)



Day after emerging from the soil

Fig. 2.7. The concentration changes of proline, alanine, total carbohydrates, and total lipids in the hemolymph for summer generation female beetles.

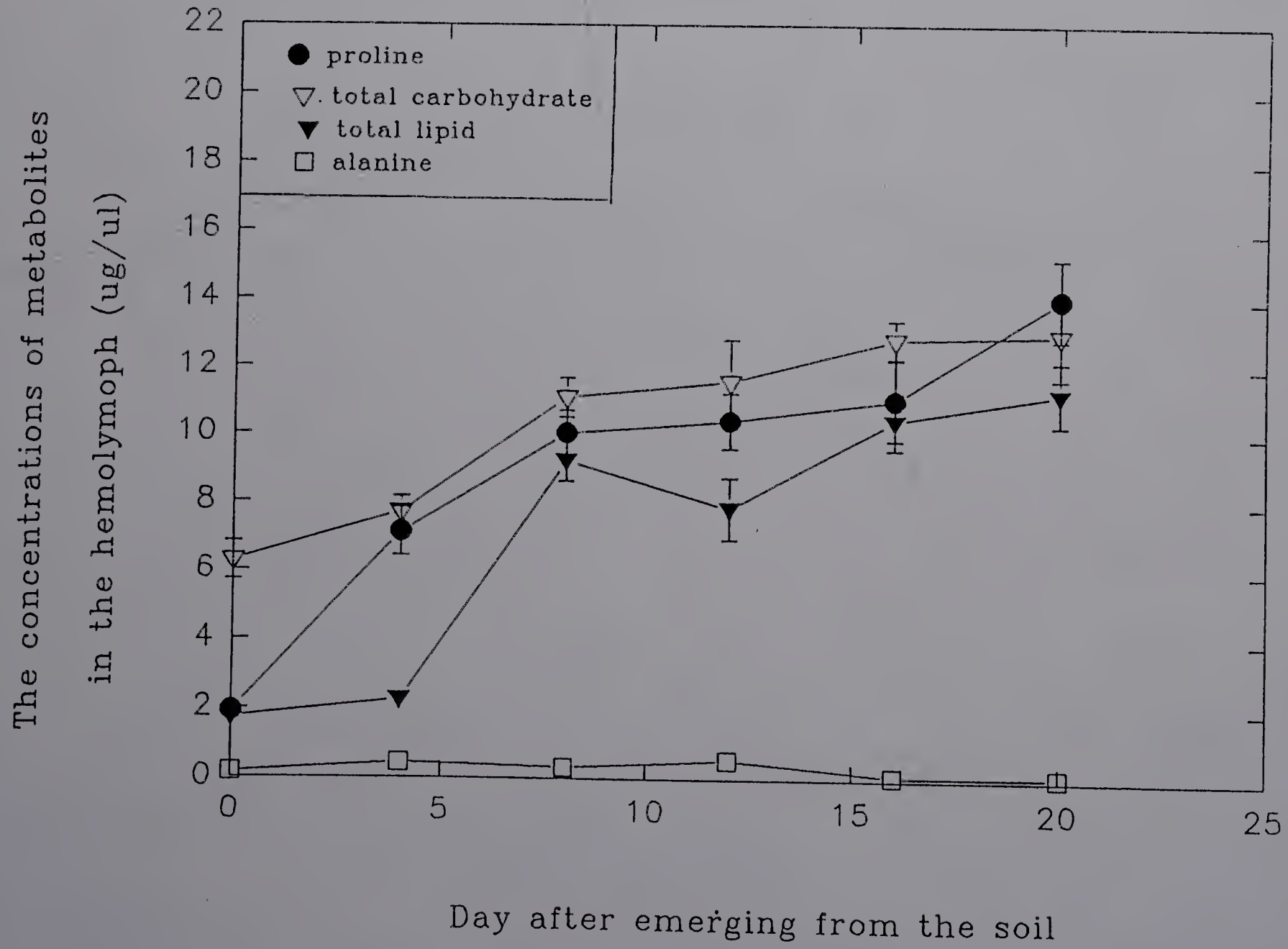

The concentrations of metabolites
in the hemolymoph (ug/ul)
proline
total carbohydrate
total lipid
alanine
0
2
4
6
8
10
12
14
16
18
20
22
0
5
10
15
20
25
Day after emerging from the soil

Fig. 2.8. The concentration changes of proline, alanine, total carbohydrates, and total lipids in the hemolymph during flight for post-diapause fed female beetles.

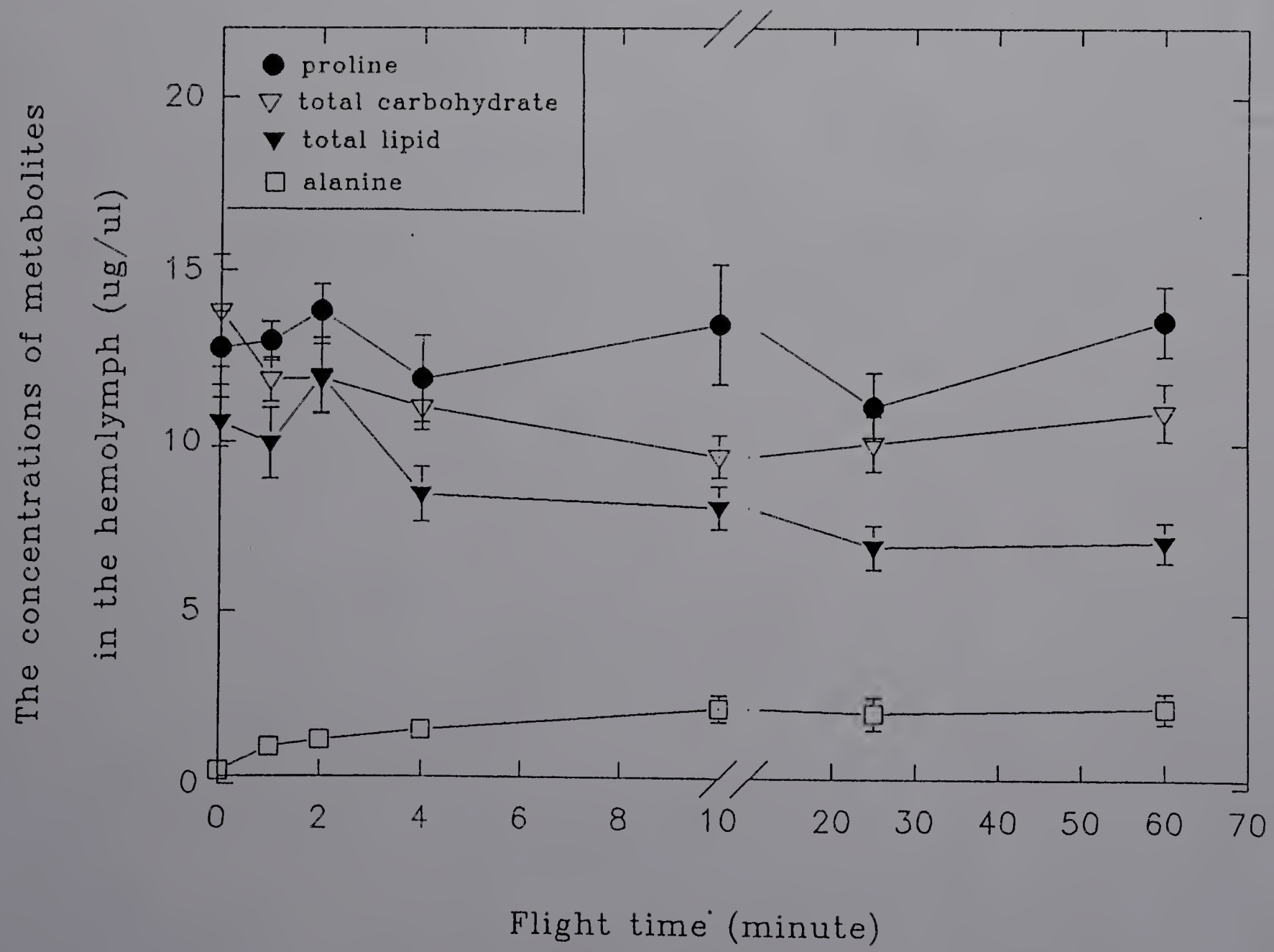

proline
total carbohydrate
total lipid
alanine
20
15
10
5
0
0
2
4
6
8
10
20
30
40
50
60
70
The concentrations of metabolites in the hemolymph (ug/ul)
Flight time (minute)

Fig. 2.9. The concentration changes of proline, alanine, total carbohydrates, and total lipids in the hemolymph during flight for post-diapause unfed female beetles.

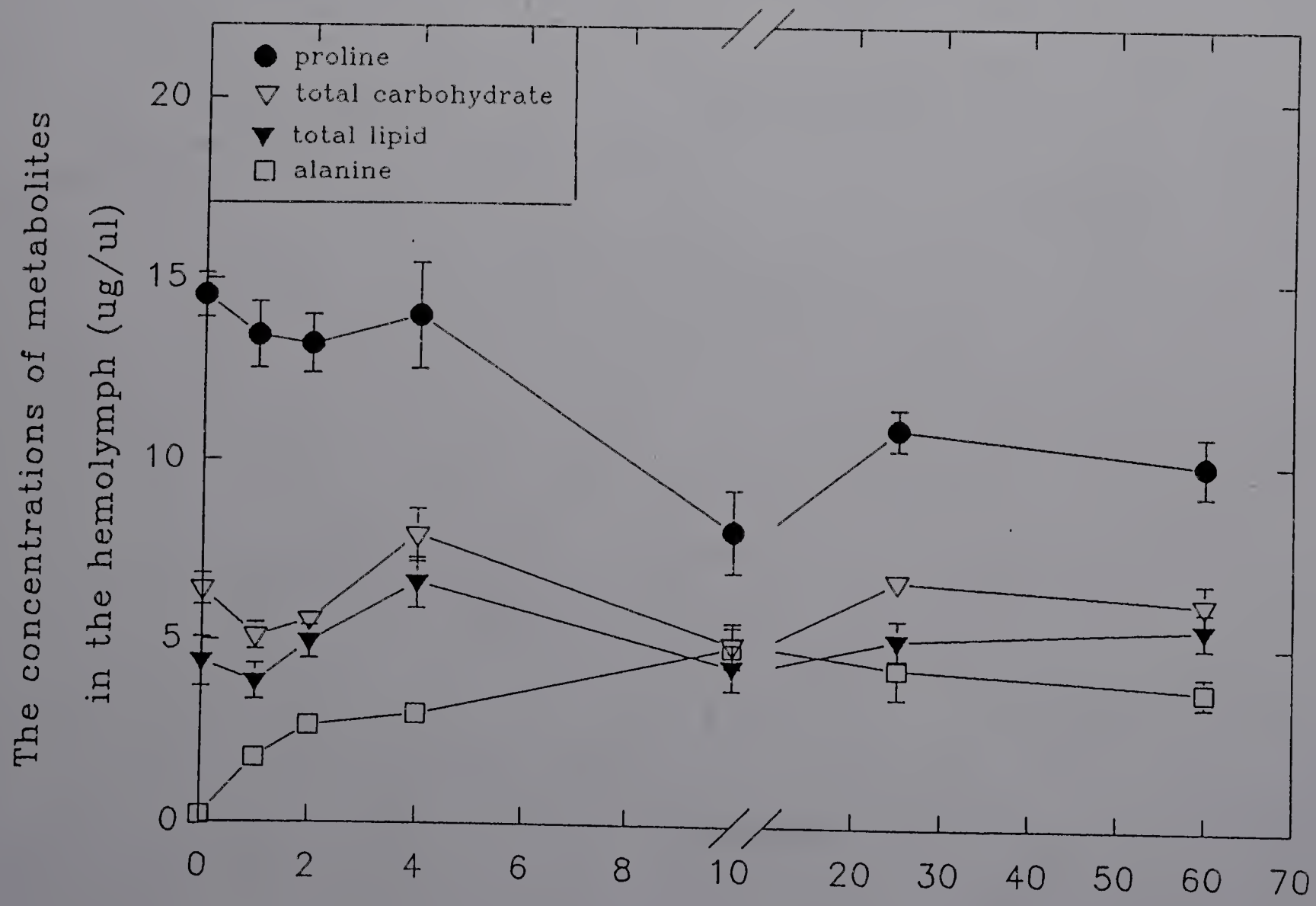
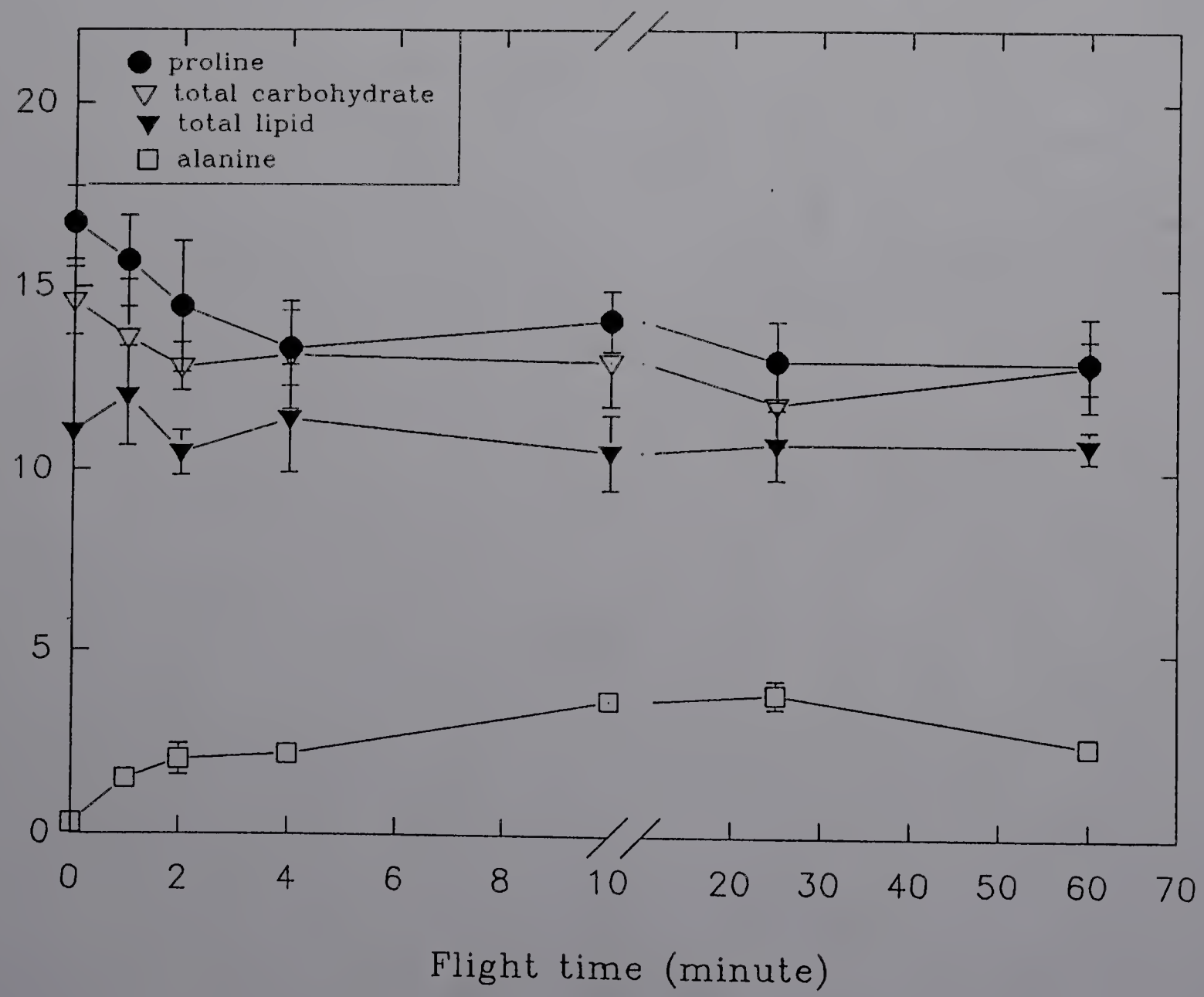

proline
total carbohydrate
total lipid
alanine
20
15
10
5
0
0
2
4
6
8
10
20
30
40
50
60
70
The concentrations of metabolites in the hemolymph (ug/ul)
Flight time (minute)

Fig. 2.10. The concentration changes of proline, alanine, total carbohydrates, and total lipids in the hemolymph dring flight for summer generation beetles.

Figure 56. Concentrations of metabolites in hemolymph (ug/ul)



CHAPTER 3

DYNAMICS OF CORPORA CARDIACA EXTRACTS IN COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY)

Introduction

Flight muscles of most insects only contain enough fuel to supply energy needs for a few minutes of flight (Beenakkers, 1984). To maintain a high metabolic rate for a long range flight, it is necessary to mobilize the fuel from the fat body. This process is usually controlled by hormones released from the insect corpora cardiaca (CC) (Orchard, 1987; Gäde, 1990).

In the Colorado potato beetle, proline and carbohydrates are used as the main substrates for flight in long-day conditioned summer generation beetles (Weeda *et al.*, 1979; Brouwers and De Kort, 1979). The hormonal factors also have been found in the CC of Colorado potato beetles that stimulate proline synthesis in the fat body and increase glucose concentration in the hemolymph (Weeda, 1981). Two of these hormonal factors have been

identified as eight amino acid peptides whose sequences are exactly the same as those found in the American cockroach (Gäde and Kellner, 1989). According to the new system of nomenclature for insect peptides (Raina and Gäde, 1988), these two hormones are Pea-CAH-I and Pea-CAH-II.

The objectives of this study were to investigate age-related changes of the extracts of corpora cardiaca (hormonal factors) activity under different conditions and to determine the age specific response of summer generation beetles to these hormonal factors.

Materials and Methods

Donors of corpora cardiaca. Overwintered female Colorado potato beetle adults were collected from the field in South Deerfield, Massachusetts in late April of 1992, before beetles emerged from soil. These beetles were sterilized with 5% bleach solution, placed in ventilated plastic boxes covered with about 10 ml of pasteurized potting soil and placed in a growth chamber maintained at 27°C and a 16:8 (L:D) long-day photoperiod until they emerged. After the beetles emerged from soil, female beetles were either fed fresh potato foliage and water or left unfed (water only). These beetles were kept in the same boxes and the same growth chamber at

27°C and a 16:8 (L:D) long-day photoperiod. The corpora cardiacae were removed from beetles on day 0, 4, 8, 12, 16, and 20 after emergence.

First summer generation female Colorado potato beetles were reared from last instar larvae collected from a potato field in South Deerfield, Massachusetts in late June, 1992. These larvae were placed on potato plants in a greenhouse maintained at 25-28°C and 16:8 (L:D) photoperiod until the larvae burrowed into the soil to pupae. When the adult beetles emerged from the soil, female beetles were placed in ventilated plastic boxes, provided fresh potato foliage and water and kept in a growth chamber maintained at 27°C and 16:8 (L:D) photoperiod. The corpora cardiaca were removed from beetles on day 0, 4, 8, 12, 16, and 20 after emergence.

Recipients of corpora cardiaca extract. Four large cages (3x3x2m) were setup in the field in Southern Deerfield, Massachusetts. Potatoes were planted in the cages. In late June of 1992, the last instar larvae were collected from an adjacent potato field and transferred into the cages. After the larvae dropped to the ground and burrowed into the soil, the potato plants and any weeds were removed from each cage. The newly-emerged beetles were collected and taken to the laboratory every morning about 10 am. Female beetles were separated and placed in an environmental chamber maintained at 27°C and

16:8 (L:D) photoperiod. Fresh potato foliage and water were provided daily. Eight to 9 day old female adults were used as recipients for extracts .

Preparation of corpora cardiaca. Colorado potato beetles were dissected in *Leptinotarsa* ringer solution (131 mM KCl, 2 mM NaCl, 1mM $CaCl_2$ and 5 mM $MgCl_2$, as described by Dortland and De Kort, 1978). The mentum was removed and the corpora cardiaca were separated carefully from the corpora allate (CA). The glands were stored in *Leptinotarsa* Ringer solution at -20°C. The CC extracts were prepared by freezing and thawing the CC in Ringer solution for 6-7 times, then centrifuged for 5 minutes at 800 g, discarded the pellet (Holwerda *et al.*, 1977).

Hemolymph sampling. Eight-9 day old recipients were injected with the corpora cardiaca extract at the base of the right hind wings by using a 10 ul syringe. The amount of 2 ul Ringer solution which contained 0.1 pair CC extract was injected into each beetle. After 45 min incubation, the left fore wings and hind wings were cut off, and hemolymph samples were taken by placing a micropipett on wounded wing base. Some crystals of phenylthiourea were added into the hemolymph to inhibit the tyrosinase. The hemolymph samples were centrifuged at 1200 g for 5 min. The cell-free hemolymph samples

were used to analyze total carbohydrate and alanine concentrations.

Total carbohydrate determination. Total carbohydrate concentrations were determined using the method described by Dubois *et al.*(1956). Phenol was utilized as the specific organic color-developing agent. This method is relatively simple and sensitive, and it was largely unaffected by the presence of protein. One ul of cell-free hemolymph was dissolved in 199 ul distilled water and then 5 ul of the phenol reagent (80% by weight, prepared by adding 2 g of distilled water to 8 g of redistilled, reagent grade phenol) was added. Then 500 ul of concentrated sulfuric acid was rapidly added. After the sample had stood at room temperature for 30 minutes, the color remained stable for several hours. During this time the optical density was determined at 485 nm. All of the samples were assayed in triplicate. Pure D-glucose crystals were dissolved in distilled water as the standard.

Since the heat required for color development was provided by the exothermic reaction of sulfuric acid and water, it was desirable to add the acid rapidly and directly onto the surface of the water. The type of test tube used was a very important factor in reducing experimental variations. Borex (12 x 75 mm) test tubes

were used in this experiment to permit good mixing and the same dissipation of heat in the all directions.

Alanine concentration determination. Alanine determination was done by generally following the methods of Willamson (1970). L-alanine is oxidized to pyruvate and ammonia in the presence of NAD^+ and alanine dehydrogenase.

L-Alanine + NAD^+ + H_2O ---------->

Pruvate + NADH + NH_4^+

Hemolymph was deproteinized with methanol not perchloric acid. Two ul of cell-free hemolymph was dissolved in 60 ul of a 60% methanol solution in 1.5 ml plastic test tubes. The contents of each tube were mixed and centrifuged at 800 g for 10 minutes. The supernatant was transferred into one clear tube, and the solution was dried under a gentle stream of air. Five hundred ul of distilled water was added into the tube and mixed. This was the sample solution. After these deproteinized procedures, 250 ul of sample solution was pipetted into a 0.5 ml cuvette. Then 250 ul hydrazine/Tris buffer (Tris, 40 mmol/l; hydrazine, 1 mol/l; EDTA, 1.4 mmol/l; pH 9.0) was added into the cuvette. The amount of 25 ul NAD solution (β-NAD, 24 mmol/l) was added. The contents of the cuvette were mixed and absorbance (A_1) was determined at the wavelength of 365 nm. Two and half ul of alanine dehydrogenase (150 KU/l) was pipetted into cuvette and

mixed. The absorbance was measured at 40, 50, and 60 minutes to determine the final absorbance (A_2) at 365 nm wavelength. The alanine concentration was calculated by using ΔA (A_2 - A_1) in a standard curve equation. Pure alanine was used to make the standard curve. Bovine serum albumin (BSA) was added to pure alanine solutions to make 10 mg/ml of the final protein concentration (about equal to the protein contents in hemolymph of Colorado potato beetle). The standard alanine solutions were deproteinzed with methanol (final concentration was about 60%). The standard curve was established for each alanine determination.

Results

Beetle response to age-depend corpus cardiacum

When the CC extracts from the post-diapause fed female beetle were injected, the day 0, day 4, day 8 CC extracts did not induce any concentration changs of the total carbohydrate in the hemolymph of recipient beetles. However, the day 12 and day 16 CC extracts induced an signifcant increase in total carbohydrate concentration (Table 3.1). The extracts of 4, 8, 12 and 16 day-old CC significantly decreased alanine concentration in the recipient beetles (Table 3.1). This data indicate that the hormonal factors in CC extracts at 12 and 16 day old

overwintered fed beetles induce synthesis or mobilization of carbohydrates as an energy sources, while the hormonal factors stimulated the proline synthesis soon after emergence (4 day old) through day 16.

The CC extracts from unfed post-diapause females have no activity in mobilizing carbohydrate during the experimental period of 20 days (Table 3.2). However, the unfed beetle CC extracts from 4 to 16 days significantly decreased the alanine concentration in recipient beetles (Table 3.2). This suggests that the hormonal factors in unfed overwintered beetles only stimulated proline synthesis, and had no effect on carbohydrate synthesis or mobilization.

The CC extracts from summer generation 8 to 20 day-old beetles induced both an increase in total carbohydrate and a decrease in alanine concentration in hemolymph of recipient beetles (Table 3.3). Therefore, the hormonal factors in CC extracts from summer beetles seemed to be the same as those in the CC extracts from overwintered fed beetle.

Response of different age summer beetle to CC extract injection

When 8 and 16 day-old beetle CC extracts from summer generation beetles were injected into 0 to 20 day-old summer generation beetles, the CC extracts of 8 day-old induced some increase of total carbohydrate

concentrations in 12 and 16 day-old recipient beetles. But this increase had no statistical difference at 0.05 level (Mann-Whitney test, $p=0.07$), while compared to the control and Ringer injected beetles. The CC extracts of 16 day-old beetles induced a significant increase in total carbohydrate concentrations in 12 to 16 day-old recipient beetles comparing with the controls ($p<0.05$) (Fig. 3.1).

This indicated that 12 to 16 day-old summer generation beetles were most responsive to the hormonal factors which were used to regulate the release and mobilization of carbohydrates as energy supplies. And CC extracts from 16 day old summer generation beetles seemed to have higher hormonal activity than those of 8 day beetles.

Discussion

This study examined the dynamic activities of CC extracts from post-diapause and summer generation beetles of different ages. My results showed that CC extract activities had a positive correlation with beetle flight abilities. The behavioral studies by Ferro *et al.* (1991) showed that the post-diapause, unfed female beetles had the highest flight abilities during 5 to 15 days after beetles became active, and the post-diapause, fed female beetles flight activity was greatest from day 11 to 20

after the beetles emerged from the soil. In this experiment, I found that CC extracts of post-diapause, unfed female beetles had the highest proline mobilization from day 4 to day 16 after beetles became active, and CC extracts from post-diapause, fed female beetles had high carbohydrate mobilization activity from day 12 to day 16 after beetle emergence, and they had high proline mobilization ability from day 4 to day 16. How to explain this correlation is still not clear. Maybe the hormonal neuropeptides in beetles associate the flight behavior. However, in locust the AKH neuropeptides can not elicit and extend the flight (Goldworthy, 1979).

Figure 3.2 summarizes the results and some of my hypotheses from Chapter 2 and Chapter 3 on energy sources used and hormonal regulation for flight by Colorado potato beetles. If the post-diapause female beetles find host plant to feed, the stores of glycogen and fatty acids in the fat body and the concentrations of carbohydrates, lipids, and proline in hemolymph increase to very high levels. When beetles take off to fly, some neuropeptides are released from corpora cardiaca to stimulate the production of proline and glucose (or trehalose) in the fat body to support the energetic needs of the flight muscles. Lipids may be a sources of energy for post-diapause fed beetles. And hormones from these post-diapause fed beetles have high activities to

mobilize carbohydrates and proline. If post-diapause female beetles do not find host plants, after starving for several days, the carbohydrate and lipid contents in the hemolymph decrease to very low levels, while the proline level remained stable. When these unfed beetle fly, proline is used as the sole fuel source. At this moment, the hormonal neuropeptides in unfed beetles can stimulate the production of proline, however, it does not have the capability to mobilize carbohydrates. So far, two peptides have been identified from Colorado potato beetle CC extracts (Gäde and Kellner, 1989). The preliminary experiments with these two peptides demonstrated a 2 to 3 fold increase in proline synthesis *in vitro* compared to controls (Gäde and Kellner, 1989). The functions of these two peptides may be different, one could mobilize proline and carbohydrates, and the other could only mobilize proline; or the different proportions of these two peptides could regulate the different fuel utilization.

Unfed post-diapause beetles fly sooner and longer than fed post-diapause beetles (Ferro *et al.*, 1991). One hypothesis is that the lower levels of carbohydrates and lipids can work as a messenger to induce the release of certain factors from corpora cardiaca. These factors then only stimulate proline mobilization. While high levels of carbohydrates and lipids are present in the

hemolymph, another factor is released to produce a different energy supply (proline and carbohydrates).

One of the objectives of this experiment was to determine the age specific response of summer generation beetles to these hormonal factors. However, the bioassay which was used in this study was not sensitive enough, it was hard to tell when summer beetles begin to respond to these metabolic neuropeptides. The results indicated that 16 day old beetles contained a higher activity of hormonal factors than 8 day old beetles, Further, 12 to 16 day old summer generation beetles had the highest ability to respond to the hormonal factors. How to explain this is still not clear.

The approach to demonstrate the metabolic neuropeptides in the central nervous system and corpora cardiaca in most studies is to use bioassays (as in my studies). The bioassay always has big variations and may not be sensitive enough to reveal subtle changes in hormone titer. Another method involves using immunochemical techniques. This became available after the peptide sequences were elucidated. However, for the whole AKH/PCHP family, because these peptides were too small (only eight to ten amino acids) and also were blocked at both terminals, it is difficult to elicit a good immunogenic response. Some analogues to the native

peptides were synthesized with free ammonia- or carboxyl terminals for effective conjugation to carrier proteins (Schooneveld *et al.*, 1983). In some cases, a tyrosine residue was added for efficient ^{125}I radiolabelling when the peptide was used to develop a radioimmunoassay (Moshitzky *et al.*, 1987; Hekimi and O'Shea, 1989). Thus far, about seven analogues were designed for Lom-AKH-I and other peptide hormones. For Colorado potato beetles, no such study has been carried out so far. However, the two hormones in Colorado potato beetles, Pea-CAH-I (pGlu-Val-Asn-Phe-Ser-Pro-Asn-TrpNH_2) and Pea-CAH-II (pGlu-Leu-Thr-Phe-Thr-Pro-Asn-TrpNH_2), can be synthesized without the -NH_2 at the N terminus, then these peptides can be conjugated to carriers (such as polylysine) at the N terminus. Subsequently more effective polyclonal antibodies against the native hormones, not the analogues, will be produced.

Table 3.1. Changes of total carbohydrate and alanine concentrations in the hemolymph of summer recipient beetles after injection of 0.1 pair of corpus cardiacum equivalents from different age overwintered fed beetles

Injection of CC extracts from post-diapause fed beetles	Response of recipient beetles	
	Carbohydrate	Alanine
--	10.44±0.53	0.55±0.06
Ringer	12.84±0.55	0.53±0.02
0 day CC extracts	14.42±0.54	0.46±0.01
4 day CC extracts	13.45±0.36	0.41±0.03*
8 days CC extracts	11.99±0.30	0.42±0.02*
12 days CC extracts	16.69±1.00*	0.41±0.02*
16 days CC extracts	17.20±1.40*	0.38±0.01*
20 days CC extracts	14.18±0.53	0.53±0.01

The mean values ± SE of nine beetles are expressed as ug/ul hemolymph.

*: Mann-Whitney test significant ($p < 0.05$) compared to ringer injection.

Table 3.2. Changes of total carbohydrate and alanine concentrations in the hemolymph of summer recipient beetles after injection of 0.1 pair of corpus cardiacum equivalents from different age overwintered unfed beetles

Injection of CC extracts from post-diapause unfed beetles	Response of recipient beetles	
	Carbohydrate	Alanine
	12.29±0.23	0.73±0.08
Ringer	9.35±0.58	0.54±0.07
0 days CC extracts	11.20±0.54	0.56±0.06
4 days Cc extracts	10.84±0.50	0.34±0.04*
8 days CC extracts	8.49±0.58	0.44±0.01*
12 days CC extracts	9.64±0.54	0.43±0.05*
16 days CC extracts	10.10±0.71	0.43±0.05*
20 days CC extracts	11.60±0.25	0.46±0.04

The mean values ± SE of nine beetles are expressed as ug/ul hemolymph.

*: Mann-Whitney test significant ($p < 0.05$), compared to ringer injection.

Table 3.3. Changes of total carbohydrate and alanine concentrations in the hemolymph of summer recipient beetles after injection of 0.1 pair of corpus cardiacum equivalents from different age summer generation beetles

Injection of CC extracts from summer generation beetles	Response of recipient beetles	
	Carbohydrate	Alanine
--	9.29±0.24	0.63±0.08
Ringer	9.35±0.48	0.58±0.06
0 days CC extracts	10.20±0.58	0.56±0.07
4 days Cc extracts	10.54±0.50	0.52±0.02
8 days CC extracts	12.49±0.77*	0.41±0.02*
12 days CC extracts	12.64±0.86*	0.43±0.07*
16 days CC extracts	13.10±0.98*	0.42±0.05*
20 days CC extracts	12.90±0.27*	0.44±0.04*

The mean values ± SE of nine beetles are expressed as ug/ul hemolymph.

*: Mann-Whitney test significant ($p < 0.05$), compared to ringer injection.

Fig. 3.1. The total carbohydrate concentration changes in day 0, day 4, day 8, day 12, day 16, and day 20 summer generation female adult beetles after injected the CC extracts from 8 and 16 day old summer generation female adult beetles. All these beetles were placed in an environmental chamber maintained at 27°C and a 16:8 (L:D) photoperiod.

Total carbohydrate concentration

in the hemolymph (ug/ul)

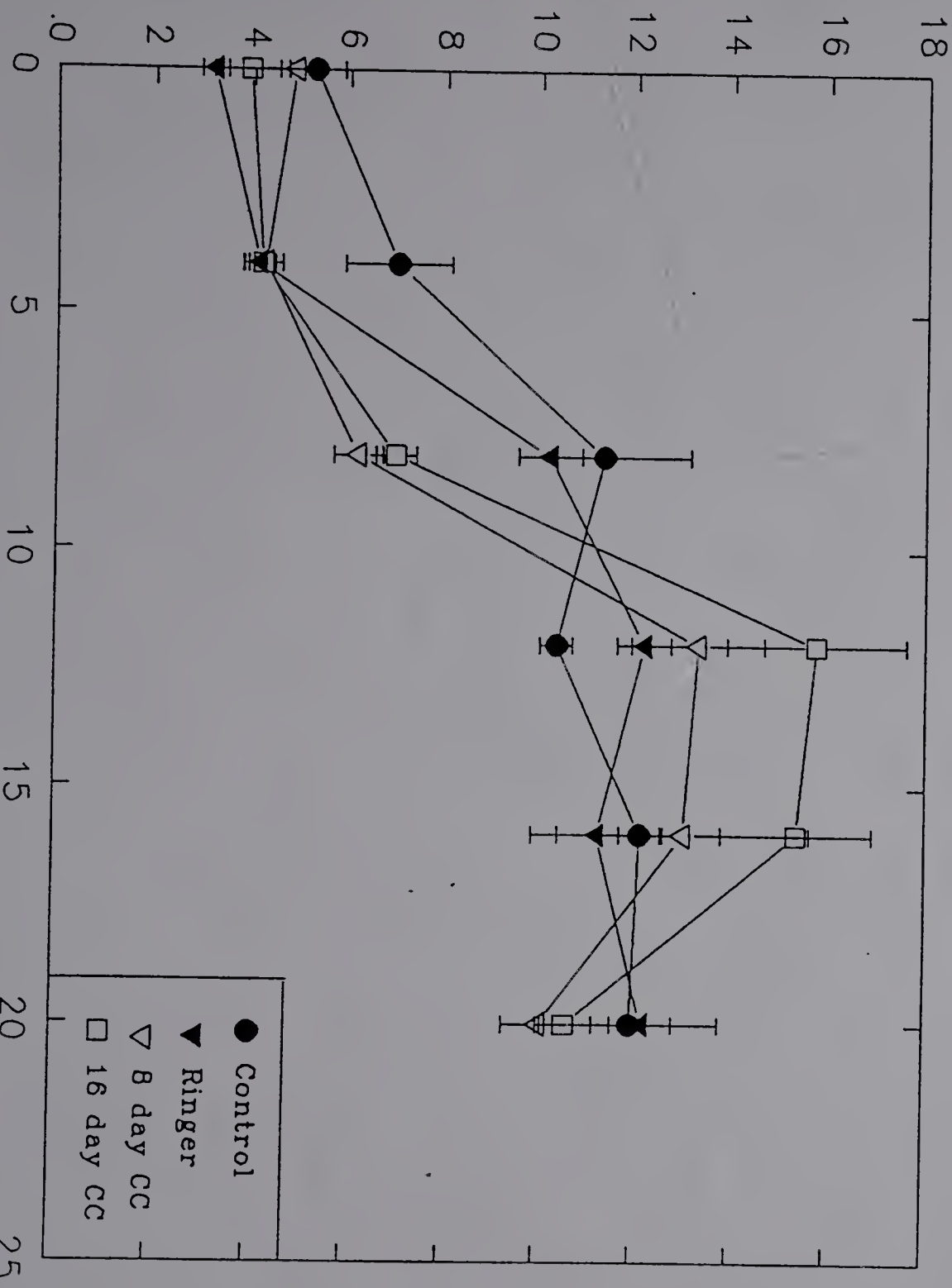
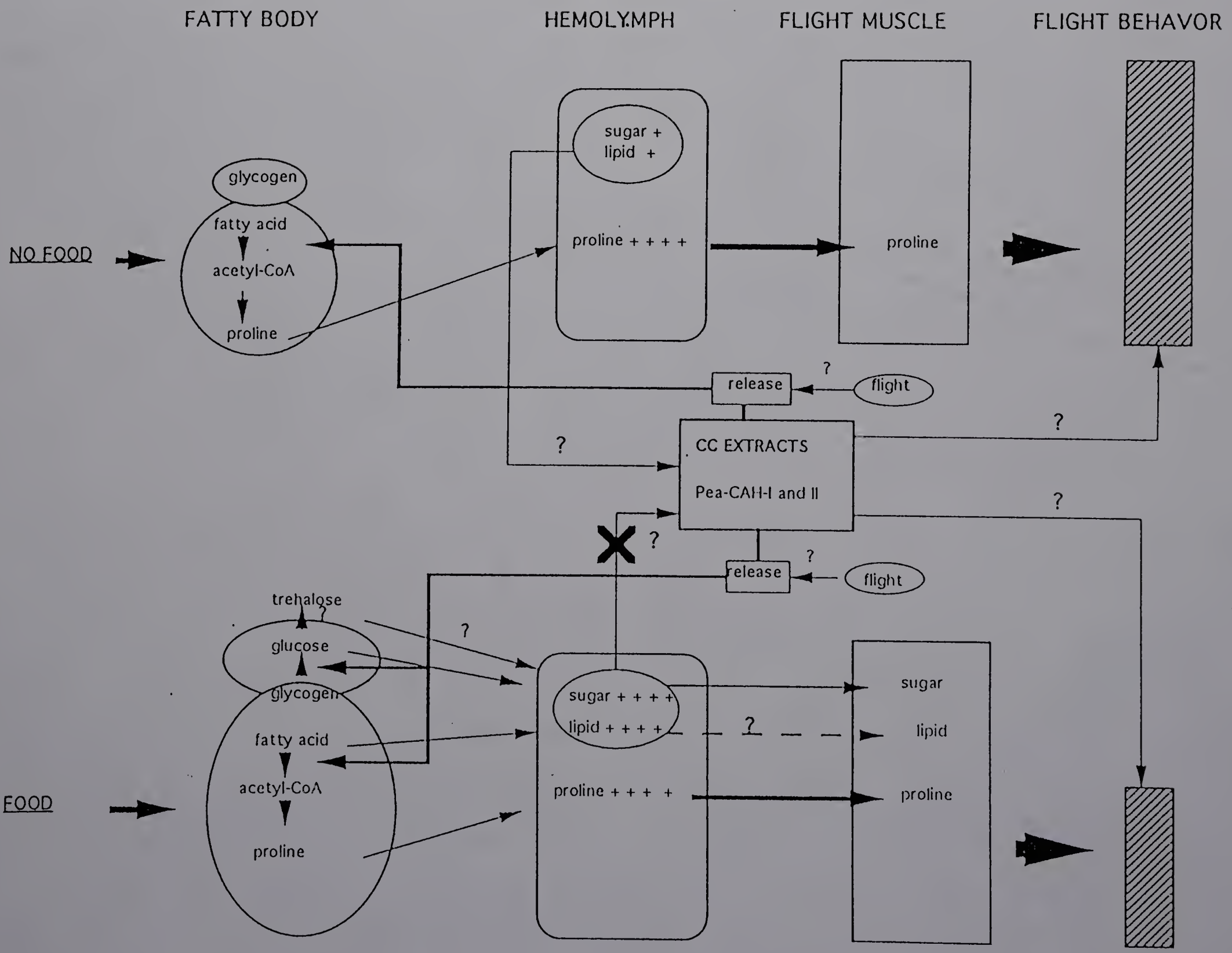


Fig. 3.2. Summary of post-diapause Colorado potato beetle energy utilization and endocrine control for flight (Please see page 61 for explain)


FATTY BODY
HEMOLYMPH
FLIGHT MUSCLE
FLIGHT BEHAVOR
NO FOOD
glycogen
fatty acid
acetyl-CoA
proline
sugar +
lipid +
proline + + + +
proline
release
?
flight
?
CC EXTRACTS
Pea-CAH-I and II
?
?
?
?
release
flight
trehalose
?
glucose
glycogen
fatty acid
acetyl-CoA
proline
FOOD
?
sugar + + + +
lipid + + + +
proline + + + +
?
sugar
lipid
proline

CHAPTER 4

FLIGHT MUSCLE DEVELOPMENT OF COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY)

Introduction

The development of flight muscles of the Colorado potato beetle has a cycle of degeneration and regeneration (De Kort, 1969). Under long-day conditions (summer generation), beetles take 7 to 9 days to complete the development of flight muscles after emergence from pupae (De Kort, 1969). Under short-day conditions (late summer and early fall), the flight muscles of newly emerged beetles develop to some extent, but the size of muscles never reach the size of flight muscles of summer generation beetles (De Kort, 1969). During diapause, the flight muscles become greatly reduced (Stegwee, 1963, 1964; De Kort, 1969).

In the spring, the flight muscles of overwintered beetles are regenerated (Stegwee, 1964; De Kort 1969). Stegwee (1964) studied the respiration of flight muscle

sarcosomes and made electron micrographs of the dorsaoventral indirect flight muscles. His results revealed that complete regeneration had occurred about the same time at which beetles were emerging from the soil. However, De Kort (1969) studied the activities of metabolism enzymes in the flight muscles of overwintered beetles and suggested that regeneration of flight muscles in overwintered beetles were similar to newly emerged summer generation beetles, i.e. post-diapause beetles took 7-9 days to regenerate their flight muscles after emerging from the soil. But Ferro *et al.* (1991) showed that some unfed or fed overwintered beetles could fly on the flight mill on the first day after emerging from the soil.

In some insects, after mating and dispersal by flight have taken place, the flight muscles are no longer required (Finlayson, 1975). In the Hymenoptera the flight muscles of queen ants are utilized as food reserves (Finlayson, 1975). As the queen may be much larger than the first brood she produces, the massive flight muscles provide a significant quantity of protein for the production of eggs and secretions to feed the larvae (Finlayson, 1975). This also occurs in the Isoptera, where the utilization of flight muscle as food reserves is important for the establishment of the new colony (Feytaud, 1912). A similar physiological link between flight muscles and egg production is seen in the

mosquitoes (Hocking 1952, 1954). Flight muscle degeneration also occurs in plant bugs (Edwards 1969; Johnson 1953), various Coleoptera (Jackson, 1933, 1952,), Diptera (Hocking, 1952) and probably in Dermaptera (Finlayson, 1975). In aquatic Hemiptera and Heteroptera reduced flight muscles are common and degeneration in adults has often been assumed to be the case (Scudder, 1971).

In the Colorado potato beetle, De Kort (1969) showed that the diameter of dorsoventral indirect flight muscles on day 12 after emerging from the soil were smaller than on day 8 under long-day conditions. However, no study has examined the regeneration of flight muscle development patterns of post-diapause beetles.

The objectives of this study were to examine the changes of flight muscle size of post-diapause beetles during and after regeneration.

Materials and Methods

Post-diapause beetles. Diapausing Colorado potato beetle adult females and males were collected in April 1993 from overwintered sites in South Deerfield, Massachusetts. At this time, the temperature in the soil was below 8 to 10°C, the threshold temperature of development for Colorado potato beetles. These beetles

were sterilized with a 5% chlorine bleach solution and covered with pasteurized potting soil, and placed in a cold room maintained at 4°C until the initiation of the experiment. Beetles were placed in an environmental chamber held at 27°C and a 16:8 (L:D) long-day photoperiod. The base temperature for determining the degree-day (DD) of flight muscles development in this study was 10°C. Beetles were sampled at 0, 50, 100, 150, 200, and 250 DD. After emerging from the soil, the beetles were either fed potato foliage or left unfed. Both groups were provided water and were reared in an environmental chamber maintained at 27°C and 16:8 (L:D) photoperiod. These post-diapause beetles were sampled at day 0 (0 DD after emerging from soil), day 10 (170 DD), day 20 (340 DD), day 30 (510 DD), day 40 (680 DD), day 50 (850 DD), and 60 day (1020 DD).

Summer generation beetles. Newly-emerged adult females and males were reared in the laboratory from egg to adult and held at 25-29°C and a 16:8 (L:D) photoperiod. These beetles were physiologically and morphologically similar to summer generation beetles. These beetles were examined to determine if there was any relationship between reprodution status and flight muscle development. Adult males and females were reared together, and allowed to mate and females produced eggs after approximately 7-8 day. Some adult males and females were reared separately to prevent mating and

females did not lay fertile eggs. The male and the female beetles from each group were dissected after 0, 10, 20, 30, 40, 50, and 60 days of development.

To prepare the flight muscles for measurement, whole beetles were fixed with Bouin's solution (Saturated picric acid aqueous: 75 ml, concentrated formalin: 25 ml, glacial acetic acid: 5 ml; Humason, 1967). After fixation, they were stored in 70% alcohol until dissections were made. The beetles were dissected in 70% alcohol under a dissecting microscope. The dorsal longitudinal muscles were measured with an ocular micrometer in the microscope. The degenerated flight muscle looked like very thin cuboid, while the fully developed flight muscle was thick cylindrical. The volume of each bundle of muscle fibers was approximated by calculating the volume of muscle column. The muscles in 10 to 15 beetles were measured for each stage.

Results

The emergence of post-diapause beetles

Fig. 4.1 was a summary of the cumulative male-female emergence of beetles relative to accumulated heat. There was no difference in the emergence of male and female beetles. Some beetles emerged from the soil after only 50 DD, while other did not emerged untill 250 DD had

accumulated. About 50% beetles emerged after 150 DD. These results were similar to the finding by Ferro *et al.* (1991), and compared with about 90 DD for the 50% accumulative emergence of Colorado potato beetle by Lashomb *et al.*(1984) in New Jersey.

The post-diapause female Colorado potato beetle needed about 150-200 DD for completing the regeneration of the indirective flight muscles (Fig. 4.2). The regeneration was a progressive process from 0 DD to 200 DD, which could be completed without food (Fig. 4.2). The flight muscle regeneration was only relative to accumulated heat that the beetle obtained, regardless whether the beetles remained in the soil or emerged from the soil (Fig. 4.2). These data supported the finding by Ferro *et al.* (1991) which showed first flight by unfed post-diapause beetle occured after 22.8 DD had accumulated. The results indicated that some beetles completed post-diapause development in preparation for dispesal by flight while they were still in the soil. Evolutionarily, these beetles that remained in the soil until their flight muscle had fully developed were not likely to die from predation or lack of host plants, and they could disperse by flight to find host plants and avoid predation. About 20% of the beetle population had their flight muscles were fully developed while still in the soil (Fig. 4.3).

Flight muscle development in Colorado potato beetle

After obtained 150-200 DD accumulated heat, most beetle flight muscles had been fully regenerated, and emerged from soil. Statistically, there was no difference for flight muscles in the males and females beetles when fed on potato foliage or left unfed. For this reason, the data for the males and females were pooled to make comparision between fed and unfed beetles.

There was no statistical difference in flight muscle volume for fed and unfed beetles until day 20. Over the 60 day observation period, there was no change in muscle volume for fed beetles while there was a rapid decline in flight muscle volume for unfed beetles. At day 60, most of the unfed beetles were dead, and the flight muscles in live beetles were reduced to the same size and same shape as those in diapausing beetles and were only 1/7 comparing with fully regenerated flight muscles (Fig. 4.3). Ferro *et al.* (1991) found that fed beetles flied less than unfed beetles up to day 20, but from day 20 to day 35 there was a rapid decline in flight by unfed beetles. This was probably due to a reduction in flight muscles by unfed beetles.

Adult male and female beetles took 7-9 days to fully develop their flight muscles for summer generation. As long as these beetles were fed potato foliage and were kept under a long-day photoperid (16:8, L:D), their

flight muscles maintained the same volume for up to 60 days. There was no significant difference of flight muscle volume between oviposited summer generation female beetles and non-oviposited female beetles (Fig. 4.4).

The flight muscles in mated and oviposited female beetles has no differnce from unmated female beetles. This suggested that mating and egg laying status did not affect the flight muscles (Fig. 4.5).

Discussion

Muscle degeneration is a very common phenomenon in insects (Finlayson 1975). Degeneration can take place either during the larval, pupal or adult stages. Most of the references on degeneration of muscles in adult insects are flight muscles (Finlayson 1975). In some insects, the volume of flight muscles is reduced during diapause. The functions of flight muscle degeneration during diapause are to provide more space in the body for the fat body to store more energy substrates, to resist low temperatures, and to reduce the energy consumption by flight muscles (Finlayson 1975).

In Colorado potato beetles, diapause coincides with a completely reversible degeneration of the flight muscles (De Kort, 1969). Before the induction of diapause, beetles engage in diapause-flights to dispersal

to diapause sites. Flight muscle degeneration must happen only after beetles disperse to diapause site. Once the beetles have located diapause sites, they no longer need to feed and do not need more space in their bodies. So the major function of degeneration flight muscle should be the reduce of energy consumption. Stegwee (1964) showed that diapausing beetles had very low respiration; compared with active beetles whose thoracic tissues compose about 80 percent of the total respiration.

In the studies by Stegwee (1964) and De Kort (1969), beetle emergence from the soil was used as the criterion in examination of flight muscle regeneration. The results from my experiments showed Colorado potato beetle in western Massachusetts needed about 150-200 degree-days to regenerate the flight muscles. My results showed that there was no difference in flight muscle development for beetles exposed to the degree-days whether they remained in the soil or emerged. For this reason, time related experiments on flight or the physiology of flight muscle based on beetle emergence as the initiation point of the experiment may need to be recalculated.

More than 50% of the post-diapause beetles emerged from the soil after 150 DD had accumulated. For the western Massachusetts, beetle emergence from the soil

seems to coincide with the completion of flight muscle regeneration in half of the population.

Diapause termination and beetle emergence from the soil are not analogous. Diapause termination occurs prior to emergence, and it is not appropriate to use beetle emergence as the index for completion of diapause development. Data from my results suggested that flight muscle regeneration could take place after diapause termination in some early emerged beetles, but in half of the population, flight muscle regeneration take place when beetles remained in the soil. However, it is uncertain that these beetles were still in diapause or not.

Temperature appears to be the most important extrinsic factor controlling post-diapause flight muscle regeneration and diapause termination. Flight muscle degeneration and regeneration also are controlled by JHIII titer in beetles (De Kort, 1969). And, JHIII titer is one factor regulating the termination of diapause in the Colorado potato beetle (Lefevere, 1989). It seems that temperature could trigger JHIII synthesis and release.

Most studies with the Colorado potato beetle during the past 35 years have been concerned with the control of diapause induction, and little attention has been given to post-diapause development (De Kort, 1990). After the completion of diapause development, CPB flight muscles

have been completely regenerated in about 200 DD (=11.8 days at 27°C). Post-diapause beetles which fed potato foliage maintained fully regenerated flight muscles for more than 60 days. Oviposition seems to have no effect on the female flight muscles. Ferro *et al*. (1991) showed that post-diapause fed female beetles could fly up to 35 days after emerging from the soil. The results from my study and Ferro *et al*.(1991) suggest that the lower levels of flight of fed beetles (local flight), while compared to unfed beetles, is a productive strategy by this generation beetles to ensure that offspring are left on host plant and distributed throughout the immediate range of host plants before beetles dispersal to other host habitats.

To maintain fully regenerated flight muscles, beetle must have a certain titer of JHIII in its hemolymph. So far, no data have established the titer of JHIII in the hemolymph necessary for the induction of flight muscle degeneration or regeneration. The rate of JHIII biosynthesis in the beetle's copora allate began to decrease in post-diapause fed beetles 10 days after emergence from the soil (De Kort *et al*., 1978; Lefevere *et al*., 1989). However, my study showed that post-diapause fed beetles could maintained the fully regenerated flight muscles for 60 days. No studies, to date, have identified the change patterns of JHIII titer for the entire life period of post-diapause beetles.

Most flight by post-diapause beetles that were not fed was within the first 20 days after beetle emerged from the soil (Ferro *et al.*, 1991). The results from my study showed beetle flight muscles for unfed beetles began to degenerate 20 days after emerged from the soil. These results are consistent with the flight mill studies of Ferro *et al.* (1991). The degeneration at the flight muscles could be due to the beetle using muscle reserve as an energy source or in preparation for entering a second season of diapause.

The Colorado potato beetles have the ability to diapause several times and diapause for more than one year without feeding (Ushatinskaya, 1976; Biever and Chauvin, 1990). No data so far is available to show the JHIII titer in these unfed beetles. And I hypotheses that JHIII titer still control the degeneration of flight muscles, maybe the injection of extra JHIII can reverse the muscle degeneration. If the degeneration of flight muscles by these beetles is to programmed for entering diapause, it is suggested the total lack of food could reduce JHIII titer and other endocrine factors in beetles, overriding the response of beetle to a long-day photoperiod and high temperatures.

Fig. 4.1. Cumulative male and female emergence of Colorado potato beetle as a function of degree-day accumulated.

06

Cumulative % emergence of post-diapause Colorado potato beetle

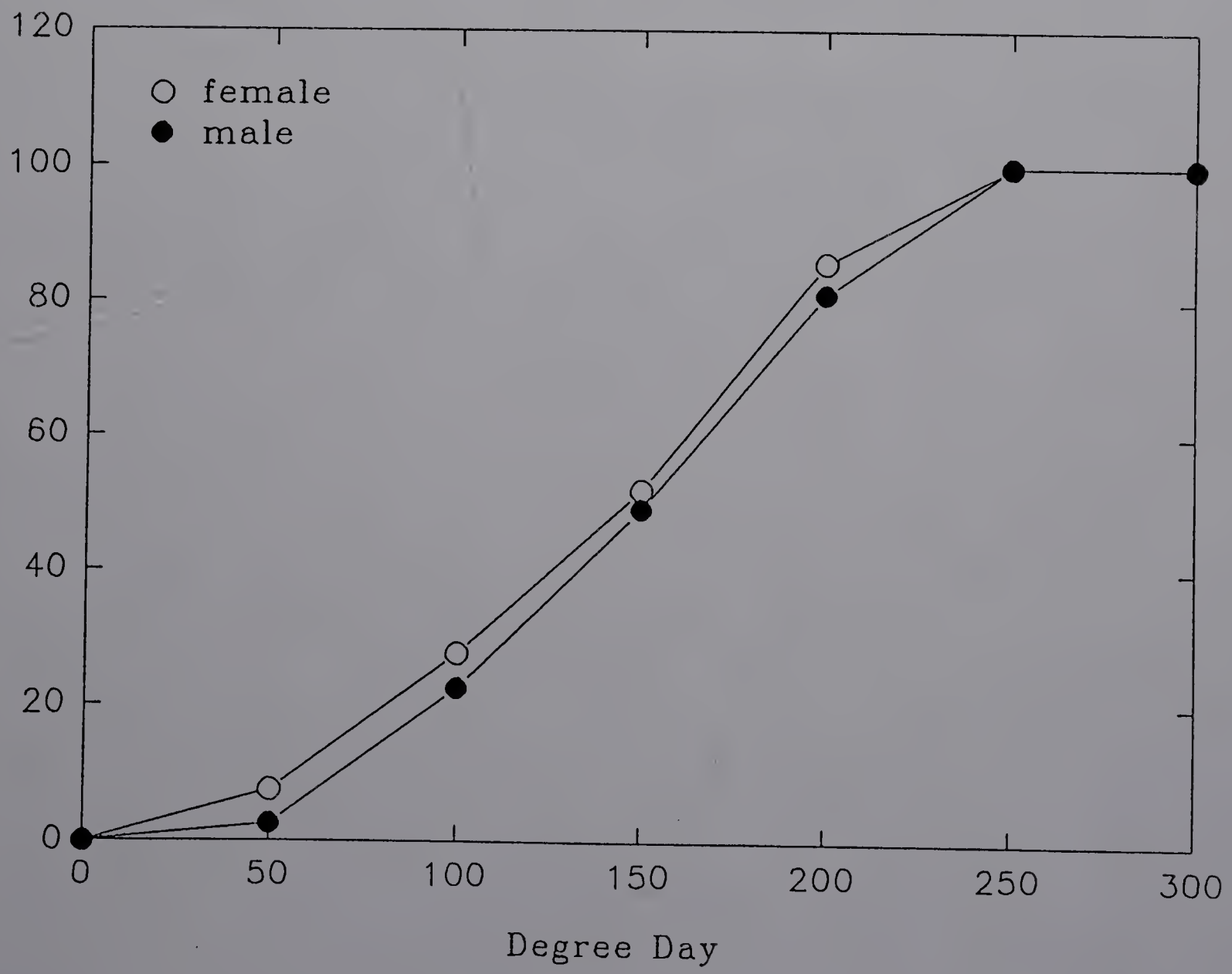


Fig. 4.2. Regeneration of flight muscles by post-diapause female Colorado potato beetles that remained in the soil or emerged from the soil. These beetles were placed in an environmental chamber maintained at 27°C and a 16:8 (L:D) photoperiod.

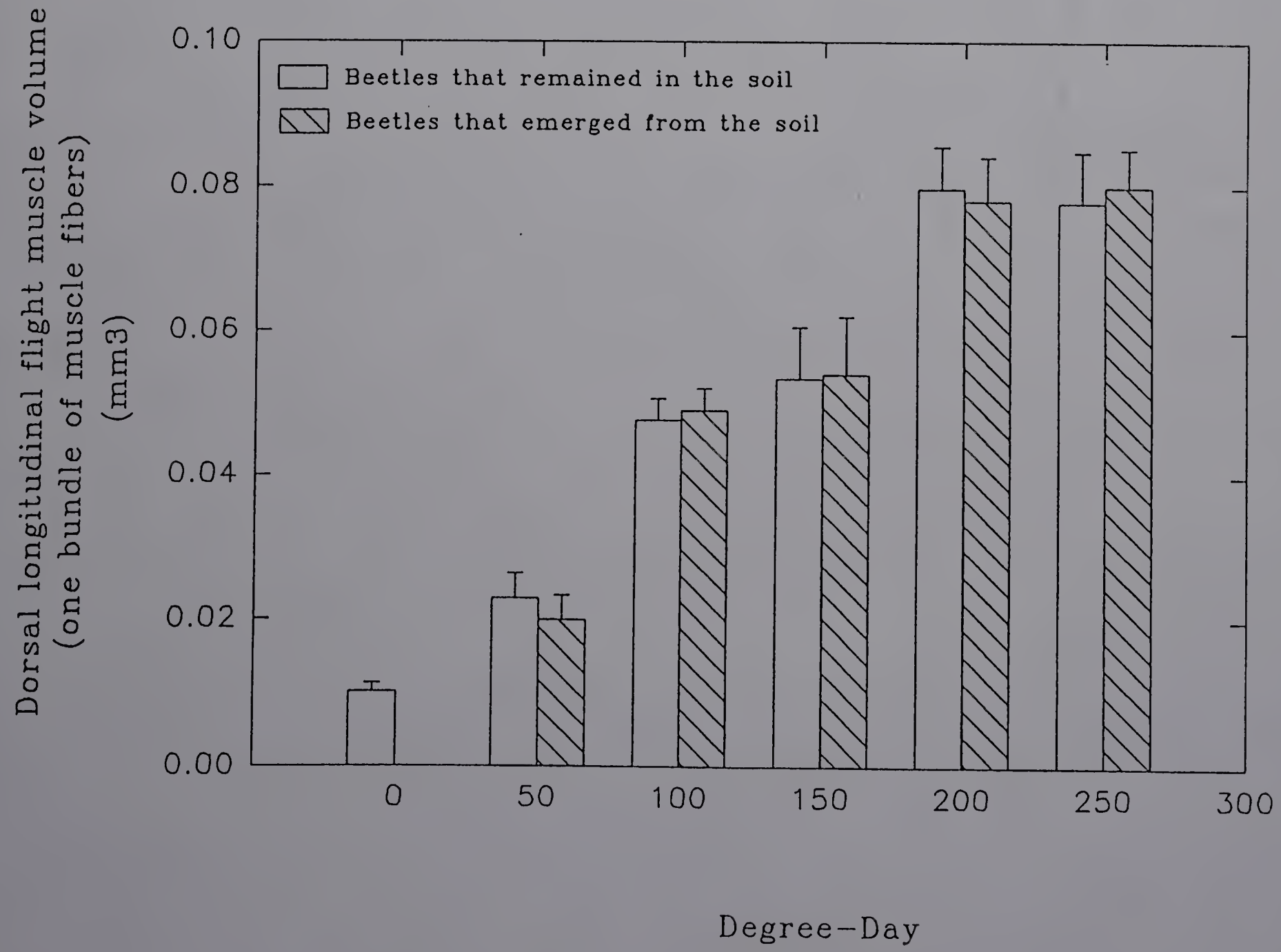

Dorsal longitudinal flight muscle volume
(one bundle of muscle fibers)
(mm3)
0.10
0.08
0.06
0.04
0.02
0.00
Beetles that remained in the soil
Beetles that emerged from the soil
0
50
100
150
200
250
300
Degree-Day

Fig. 4.3. The volume changes of the dorsal longitudinal flight muscles for post-diapause fed and unfed female Colorado potato beetles. These beetles were placed in an environmental chamber maintained at 27°C and a 16:8 (L:D) photoperiod. If calculate degree-day after emergence, 10 day = 170 DD, 20 day = 340 DD, 30 day = 510 DD, 40 day = 680 DD, 50 day = 850 DD, and 60 day = 1020 DD.

*: The volume of flight muscles are significantly different between fed beetles and unfed beetles.

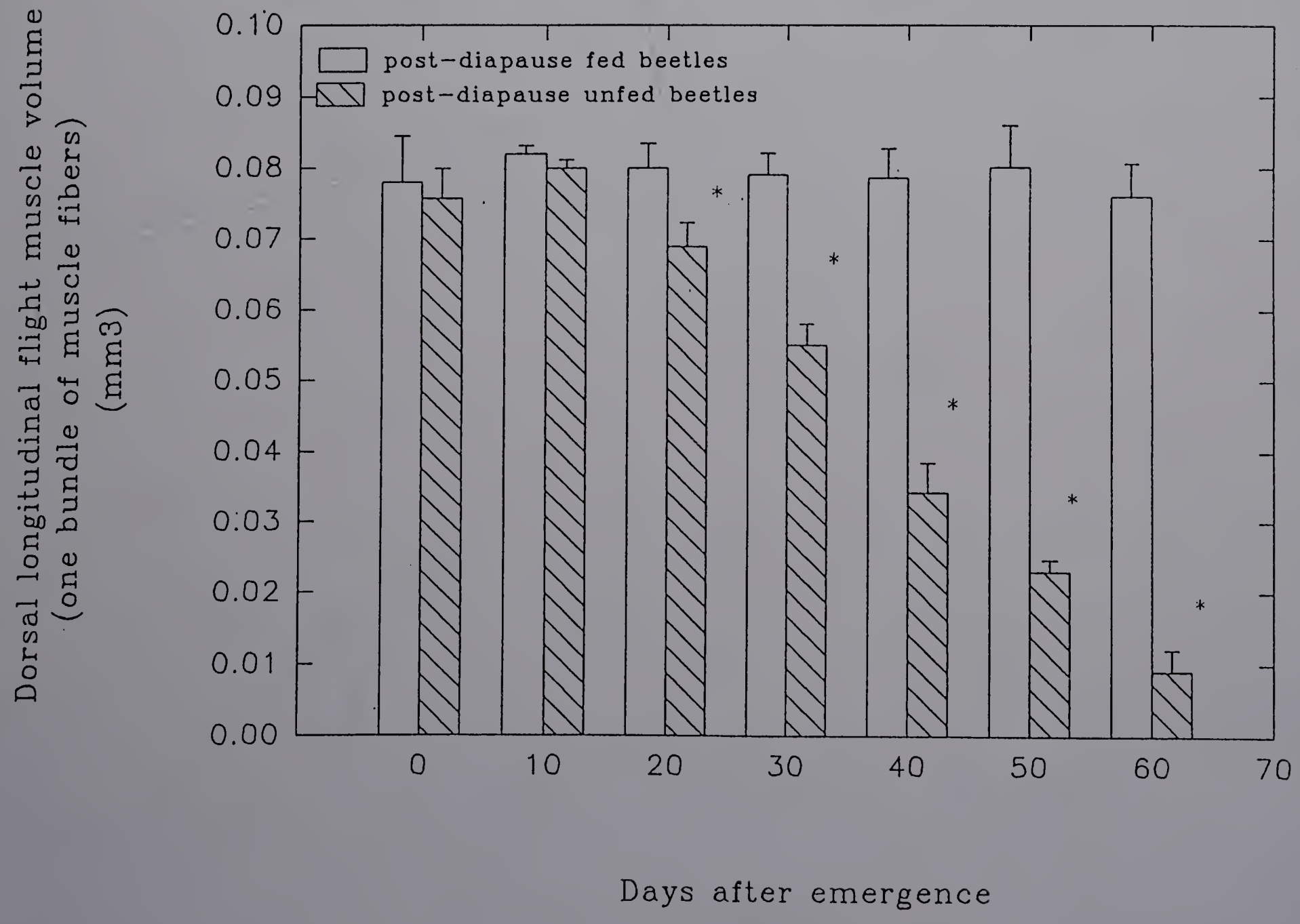

Dorsal longitudinal flight muscle volume
(one bundle of muscle fibers)
(mm3)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
post-diapause fed beetles
post-diapause unfed beetles
*
0
10
20
30
40
50
60
70
Days after emergence

Fig. 4.4. Flight muscle development of summer generation male and female Colorado potato beetles. These beetles were placed in an environmental chamber maintained at 27°C and a 16:8 (L:D) photoperiod. If calculate degree-day after emergence, 10 day = 170 DD, 20 day = 340 DD, 30 day = 510 DD, 40 day = 680 DD, 50 day = 850 DD, and 60 day = 1020 DD.

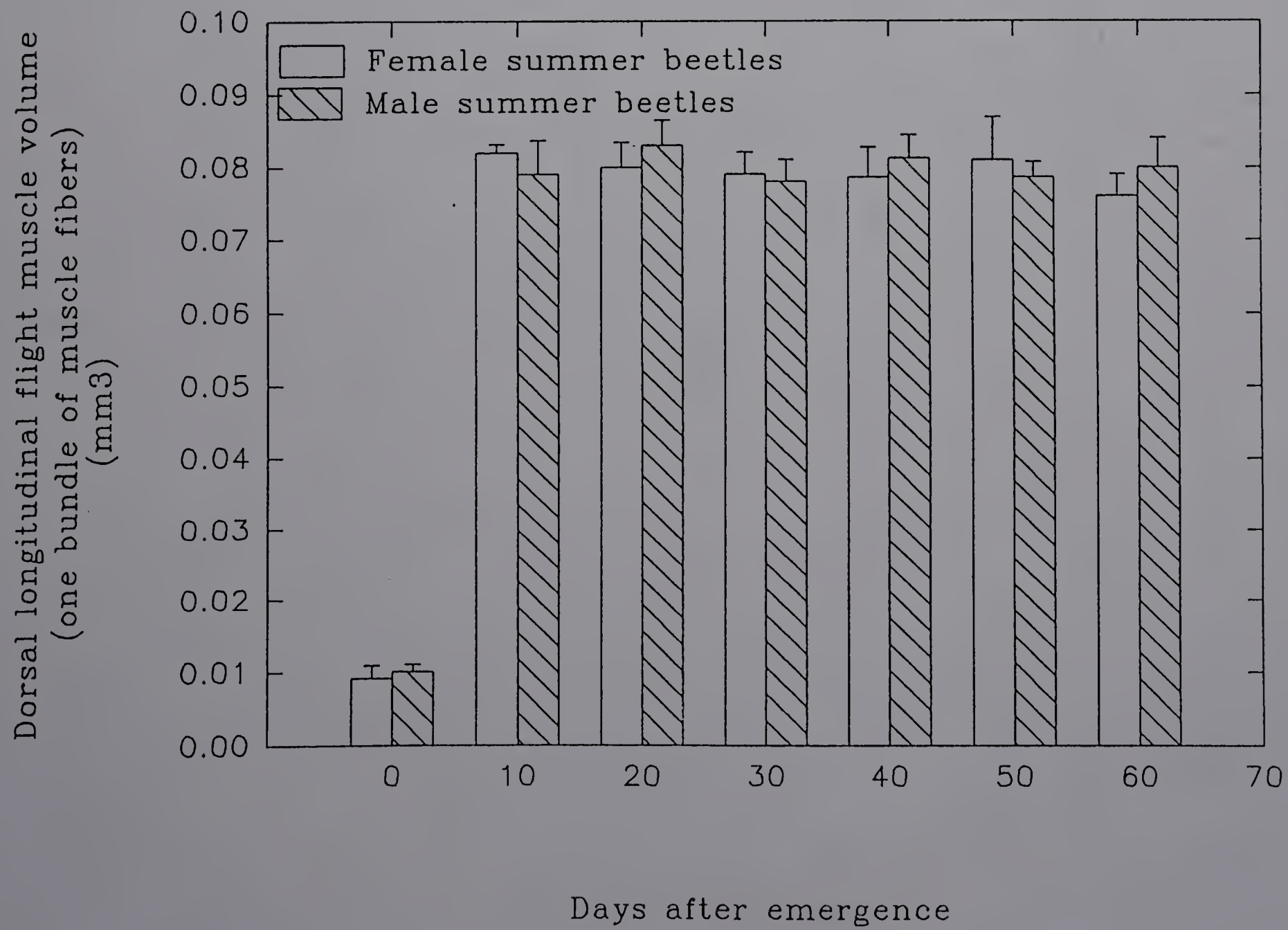
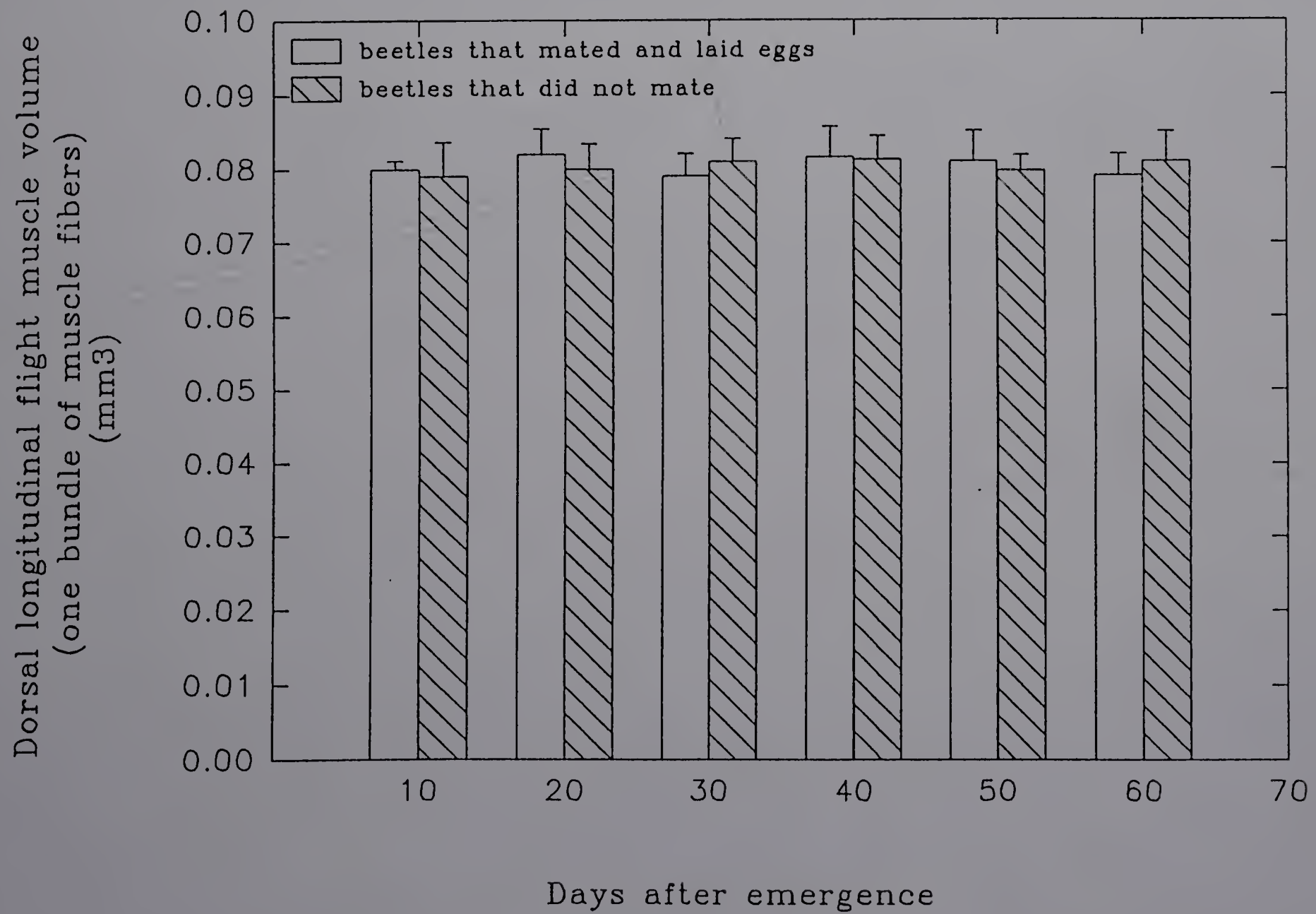

Dorsal longitudinal flight muscle volume
(one bundle of muscle fibers)
(mm3)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Female summer beetles
Male summer beetles
0
10
20
30
40
50
60
70
Days after emergence

Fig. 4.5. Flight muscle development of summer generation mated and laid eggs female and did not mate female Colorado potato beetles. These beetles were placed in an environmental chamber maintained at 27°C and a 16:8 (L:D) photoperiod. If calculate degree-day after emergence, 10 day = 170 DD, 20 day = 340 DD, 30 day = 510 DD, 40 day = 680 DD, 50 day = 850 DD, and 60 day = 1020 DD.


Dorsal longitudinal flight muscle volume
(one bundle of muscle fibers)
(mm3)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
beetles that mated and laid eggs
beetles that did not mate
10
20
30
40
50
60
70
Days after emergence

REFERENCES CITED

Beenakkers A. M. Th. 1984. Insect flight musle metabolism. Insect Biochem. 14(3): 243-260.

Bergman I. and Loxley. 1970. New spectrophotometric method for the determination of proline in tissue hydrolyzates. Analytical Chemistry. 42(7): 702-706.

Bhakthan N. M. G., and J. H. Borden, and K. K. Nair. 1970. Fine structure of degenerating and regenerating flight muscles in a bark beetle, *Ips confusus*. J. Cell Sci. 6: 807-819.

Biever K. D. and R. L. Chauvin. 1990. Prolonged dormancy in Pacific Northwest population of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptra:Chrysomelidae). Can. Entomol. 122: 175-177.

Brouwers E. V. M. and C. A. D. De Kort. 1979. Amino acid metabolism during flight in the Colorado potato beetle, *Leptinotarsa decemlineata*. J. Insect Physiol. 25: 411-414.

Bursell E. 1981. The role of proline in energy metabolism. Pp. 135-154. *In* R. G. H. Downer [ed], Energy Metabolism in Insect. Plenum Press, New York.

Candy D. J. 1989. Utilization of fuels by the flight muscles. Pp 305-319. *In* Goldsworthy and Wheeler [eds], Insect Flight. CRC Press, Inc., Boca Raton, Florida.

Cappeart D. L. 1988. Ecology of Colorado potato beetle in Mexico. Masters Thesis in Entomology, Michigan State Unversity, East Lansing.

Caprio M. A. and E. J. Grafius. 1990. Effects of light, temperature and feeding status on flight initiation in post-diapause Colorado potato beetles (Coleoptera:Chrysomelidae). Environ. Entomol. 19: 281-285.

Casagrande, R. A. 1987. The Colorado potato beetle: 125 years of mismanagement. Bull. Entomol. Soc. Am. 33: 142-150.

De Kort C. A. D. 1969. Hormone and the structural and biochemical properties of the flight muscle in the Colorado beetle. Meded Landbouwhogeschool Wageningen, 69-2.

De Kort C. A. D., A. K. M. Bartelink, and R. R. Schuurmans. 1973. The significance of L-proline for oxidative metabolism in the flight muscles of the Colorado beetle, *Leptinotarsa decemilneata*. Insect Biochem. 3: 11-17.

De Kort C. A. D., S. J. Kramer, and M. Wieten. 1978. Regulation of Juvenile hormone titres in the adult Colorado beetle: interaction with carboxylesterase and carrier protein. Comparative Endocrinology. Pp 507-510. Elsevier/North-Holland Biomedical Press, Amsterdam.

De Kort C. A. D. 1990. Thirty-five years of diapause research with the Colorado potato beetle. Entomol. Exp. Appl. 56: 1-13.

De Wilde J. and T. Hsiao. 1981. Geographic diversity of the Colorado potato beetle and its infestation in Eurasia. Pp 47-68. *In*: J. H. Lashamb and R. Casagrarde [eds], Advances in potato pest managemant.

De Wilde J., C. S. Duintjer, and L. Mook. 1959. Physiology of diapause in the adult Colorado beetle (*Leptinotarsadecemlineata*). I. The photoperiod as a controlling factor. J. Insect Physiol. 3: 75-85.

De Wilde J. 1978. Seasonal states and endocrine levels in insects. Pp 10-19. *In* Assenmacher and farner [eds], Environmental Endocrinology. Springer, New York.

Dortland J. F. and C. A. D. De Kort. 1978. Protein synthesis and storage in the fat body of Colorado potato beetle, *Leptinotarsa decemlineata*. Insect Biochem. 8: 93-98.

Dubois M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28(3): 350-356.

Edwards F. J. 1969. Development and histolysis of the indirect flight muscles in *Dysdercus intermedius*. J. Insect Physiol. 15: 1591-1599.

Ferro D.N., A. F. Tuttle, and D. C. Weber. 1991. Ovipositional and flight behavior of overwintered Colorado potato beetle (Coleoptera: Chrysomelidae). Environ. Entomol. 20(5): 1309-1314.

Ferro D. N., J. A. Logan, R. H. Voss, and J. S. Elkinton. 1985. Colorado potato beetle (Coleoptera: Chrysomelidae) temperature-dependent growth and feeding rate. Environ Entomol. 14: 343-348.

Feytaud J. 1912. Contribution a l'etude du Termite lucefuge (Anatomie, Fondation de Colonies Nouvelles). Arch Anat. Micros. 13: 481-607.

Finlayson L. H. 1975. Development and degeneration. Pp 99-149. *In* P. N. R. Usherwood [ed], Insect Muscle. Academic Press.

Gäde G. and R. Kellner. 1989. The metabolic neuropetides of thecorpus cardiacum from the potato beetle and the American cockroach are identical. Peptides. 10: 1287-1289.

Gäde G. 1990. The adipokinetic hormone/red pigment-concentrating hormone peptide family: structures, interrelationships and functions. J. Insect Physiol. 36: 1-12.

Goldsworthy G. J., W. Mordue, and J. Guthkelch. 1972. Studies on insect adipokinetic hormones. General and Comparative Endocrinology. 18: 545-551.

Goldsworthy G. J., A. R. Jutsum, and N. L. Robinson. 1979. Substrate utilization and flight speed during tethered flight in the locust. J. Insect Physiol. 25: 183-185.

Handel E.V. 1985. Rapid determination of total lipids in mosquitoes. J. Am. Mosq. Control Assoc. 1(3): 302-304.

Hare J. D. 1990. Ecology and management of the Colorado potato beetle. Annu. Rev. Entomol. 35: 81-100.

Hekimi S. and M. O'Shea. 1989. Antisera against AKHs and AKH precursors for experimental studies of an insect neurosecretory system. Insect Biochem. 19(1): 79-83.

Hocking B. 1952. Autolysis of flight muscle in a mosquito, *Aedes communis*. Nuture. 169: 1101.

Hocking B. 1954. Flight muscle autolysis in *Aedes communis*. Mosquito News. 14: 121-123.

Holwerda D. A., J. V. Doorn, and A. M. Th. Beenakkers. 1977a. Characterization of the adipokinetic and hyperglycaemic substances from the locust corpus cardiacum. Insect Biochem. 7: 151-157.

Holwerda D. A., E. Weeda, and J. V. Doorn. 1977b. Separation of the hyperglycaemic and adipokinetic factors from the cockroach corpus cardiacum. Insect Biochem. 7: 477-481.

Humason G. L. 1967. Animal tissue techniques. W. H. Freeman Company. p14.

Hurst G. W. 1969. Can the Colorado beetle fly from France to England? Entomol. Monthly Magazine, 105: 269-272.

Jackson D. J. 1933. Observations on the flight muscles of *Sitona* weevils. Ann. Appl. Biol. 20: 731-770.

Jackson D. J. 1952. Observations on the cpacity for flight of water beetles. Proc. R. Ent. Soc. Lond. (A). 27: 57-70.

Jacques, R. L. 1988. The potato beetles: the genus *Leptinotarsa* in North America. E.J. Brill, New York, pp 144.

Johnson B. 1953. Flight muscle autolysis and reproduction in aphids. Nature. 172: 813.

Johnson C. G. 1966. A functional system of adaptive disperal by flight. Annu Rev. Entomol. 11: 233

Johnson C. G. 1969. The migration and dispersal of insects by flight. Methuen, London.

Kammer A. and B. Heinrich. 1978. Insect flight metabolism. Adv. Insect Physiol. 13: 133-228.

Khan M. A. and C. A. D. De Kort. 1978. Further evidence for the significance of proline as a substrate for flight in the Colorado potato beetle, *Leptinotarsa decemlineata*. Comp. Biochem. Physiol. 60B: 407-411.

Lashomb J. H., Yuen-Shaung NG, G. Ghidiu, and E. Green. 1984. Description of spring emergence by the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleptera: Chrysomelidae), in New Jersey. Environ. Entomol. 13: 907-910.

Lefevere K. S., A. B. Koopmanschap, and C. A. D. De Kort. 1989. Juvenile hormone metabolism during and after diapause in the female Colorado potato beetle, *Leptinotarsa decemlineata*. J. Insect Physiol. 35: 129-135.

Mateeva-Radeva A. 1985. Colorado potato beetle in Bulgaria: geographical variation in population dynamics and consideration for its control, pp 139-144. *In* D. N. Ferro and R. H. Voss [eds], Procceeding of the symposium on the colordo potato beetle, XVIIth International Congress of Entomology. Mass. Agric. Expt. Sta., Res. Bull. 704, Amherst, Mass.

Mordue W. and C. A. D. De Kort. 1978. Energy substrates for flight in the Colorado beetle, *Leptinotarsa decemlineata* Say. Insect Physiol. 24: 221-224.

Moshitzky P., M. Yamashiro, D. Stuve, C. Ramachandran, and S. W. Appelbaum. 1987. Determination of locust AKH-I by radioimmunoassay and the identification of an AKH-like factor in the locust brain. Insect Biochem. 17: 765-769.

Peferoen M., R. Huybrechts, and A. De Loof. 1981. Longevity and fecundity in the Colorado potato beetle, *Leptinotarsa decemlineata*. Entom. Exp. Appl. 29: 321-329.

Price P. W. and Waldbaner. 1975. Ecological aspects of pest management, pp 37-74. In R. L. Metcalf and W. H. Luckmann [eds], Introduction to insect pest management. John Wiley and Sons, Now York.

Raina A. K. and G. Gäde. 1988. Insect peptide nomenclature. Insect Biochem. 18: 785-787.

Rankin M. A. and S. M. Rankin. 1979. Physiological aspects of insect migratory behavior, pp 35-63. In Kennedy G. G. and Rabb R. L. [eds.], Movement of high mobile insects; concepts and methodology in research. North Carolina State Univ., Raleigh, North Carolina.

Rankin M. A. and J. C. A. Burchsted. 1992. The cost of migration in insects. Annu. Rev. Entomol. 37: 533-559.

Schooneveld H., G. I. Tesser, J. A. Veenstra, and H. M. Rombergprivee. 1983. Adipokinetic hormone and AKH-like peptide demonstrated in the corpora cardiaca and nervous system of *Locusta migratoria* by immunocytochemistry. Cell Tiss. Res. 230: 67-76.

Scudder G. C. E. 1971. The postembryonic development of the indirect flight muscles in *Cenocorixa brifida* (Hung) (Hemiptera:Corixidae). Can. J. Zool. 49: 1387-1398.

Stegwee D. 1964. Respirtory chain metabolism in the Colorado potato beetle-II. Respiration and oxidative phosphorylation in 'sarcosomes' from diapausing beetles. J. Insect Physiol. 10: 97-102.

Tauber M. J., C. A. Tauber, J. J. Obrychi, B. Gollands, and R. J. Wright. 1988. Geographical variation in responses to photoperiod and temperature by *Leptinotarsa decemlineata* (Coleoptera:Chrysomelidae) during and after dormancy. Ann. Entomol. Soc. Am. 81(5): 764-773.

Tower W. L. 1906. An investigation of evolution in Chrysomelid beetles of the genus *Leptinotarsa*. Carnegie Institute, Washington D. C.

Ushatinskaya R. S. 1976. Prolonged diapause in Colorado beetle and conditions of its formantion, pp 1-27. *In* Arnoldi K. V. [ed], Ecology and physiology of diapause in Colorado beetle. Indian National Scientific Documentation Center. New Delhi,

Ushatinskaya R. S. 1978. Seasonal migration of adult *Leptinotarsa decemlineata* (Insecta, Coleoptera) in different types of soil and physiological variations of individuals in hibernating populations. Pedobiologia. 18: 120-126.

Weber D. C. 1992. Dispersal and diet of Colorado potato beetle. Ph.D dissertation. Department of Entomology, University of Massachsetts, Amherst, 159 pp.

Weber, D. C., D. N. Ferro, and J. G. Stoffolano, JR. 1993. Quantifying flight of Colorado potato beetles (Coleoptera: Chrysomelidae) with a microcomputer-based flight mill system. Ann. Entomol. Soc. Am. 86(3): 366-371.

Weber, D. C. and D. N. Ferro. 1993. Distribution of overwintering Colorado potato beetle in and near Massachusetts potato fields. Entomol. exp. appl. 66: 191-196.

Weber, D. C. and D. N. Ferro. 1994. Movement of overwintered Colorado potato beetles in the field. J. Agric. Entomol. 11(1): 17-27.

Weeda E., C. A. D. De Kort, and A. M. Th. Beenakkers. 1980a. Oxidation of Proline and Pyruvate by flight muscle mitochondria of the Colorado Beetle, *Leptinotarsa decemlineata* Say. Insect Biochem. 10: 305-311.

Weeda E., C. A. D. De Kort, and A. M. Th. Beenakkers. 1980b. Proline synthesis in fat body of *Leptinotarsa decemlineata*. Insect Bioch. 10: 631-636.

Weeda E. 1981. Hormonal regulation of prolinr synthesis and glucose release in the fat body of the Colorado potato beetle, *Leptinotarsa decemlineata*. J. Insect Physiol. 27: 411-417.

Weeda E., C. A. D. De Kort, and A. M. Th. Beenakkers. 1979. Fuels for energy metabolism in the Colorado potato beetle, *Leptinotarsa decemlineata* Say. J. Insect Physiol. 25: 951-955.

WiKtelius S. 1981. Wind dispersal of insects. Grana. 20: 205-207.

Williams C. E. 1988. Movement, dispersion and orientation of population of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleptera: Chrysomelidae)in eggplant. The Great Lakes Entomologist. 21: 31-38.

Williamson D. H. 1970. L-alanine: determination with alanine dehydrogenase, pp 341-344. *In* Bergmeyer H. U. [ed], Method in Enzymatic analysis.

Voss R. H., D. N. Ferro, and J. A. Logan. 1988. Role of reproductive diapause in the population dynamics of the Colorado potato beetle (Coleoptera: Chrysomelidae) in Western Masssachusetts. Environ. Entomol. 17(5): 863-871.

Voss R. H. 1989. Population dynamics of the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleptera: Chrysomelidae), in western Massachusetts with particular emphasis on migration and dispersal processes. PH.D Dissertation. University of Massachusetts, Amherst, pp 162.

Voss R. H. and D. N. Ferro. 1990a. Ecology of migrating Colorado potato beetles (Coleoptera: Chrysomelidae) in western Masschusetts. Environ. Entomol. 19(1): 123-129.

Voss R. H. and D. N. Ferro. 1990b. Phenology of flight and walking by Colorado potato beetle (Coleoptera: Chrysomelidae) adults in western Massachusetts. Environ. Entomol. 19(1): 117-122.